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The firebrat, Thermobia domestica (Packard), and its gregarine parasites

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THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD), AND ITS GREGARINE PARASITES

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FOREWORD

This thesis consists of three minor theses resulting from three diverse lines of investigation upon one insect species:

Part One: Methods and Observations in Rearing the Firebrat, *Thermobia domestica* (Packard)(Thysanura)

Part Two: The Temperature Relations of the Firebrat, *Thermobia domestica* (Packard)(Thysanura)

Part Three: The Gregarine Parasites of the Firebrat, *Thermobia domestica* (Packard)(Thysanura)

Each paper has its own introduction, review of literature, description of methods, statement and discussion of results, summary, and list of literature cited. The subject matters of Parts One and Two necessitate some departures from the usual formalities of presentation, such as some intermingling of statements and discussions of results; but in Part Three, which is regarded as the most important part of the work, a regular thesis form is adhered to.

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PART ONE

METHODS AND OBSERVATIONS IN REARING THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD)(THYSANURA)

METHODS AND OBSERVATIONS IN REARING THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD)(THYSANURA)

I. INTRODUCTION

A. The Use of Representative Species

Most of the existing generalizations upon the nature of living things could hardly have been attained without a systematic approach. The successive steps are: first, the recognition and definition of species; secondly, the arrangement of the species in taxonomic groups; and finally, the selection and intensive study of certain individuals as group representatives. The selection of such representatives is often governed to a great extent by their availability and their suitability for laboratory use. It is patently desirable that a representative species should be one which can readily be procured and used by many workers in various parts of the world. Moreover, where the nature of the study permits, the selected organism should be one which can be cultivated and observed under closely controlled conditions.

B. Insects as Experimental Animals

The use of insects as experimental animals in studies upon physiology, toxicology, parasitology, genetics, and other branches of animal biology, is probably increasing. The insects so employed are usually those which can be cultured continuously in the laboratory. The most outstanding example is the pomace fly, Drosophila, reared and used by geneticists; but many other insects are in frequent use, such as cockroaches, various moths and beetles infesting stored food, the silkworm, the house fly, and various parasitic wasps. It should be noted however that, with the possible exception of the cockroach, these insects represent highly specialized groups within the class Insecta, while the Apterygota, believed by some authorities to include the most generalized of insects, have not been represented in experimental studies to any material extent. It is the purpose here to call attention to an apterygotous insect which seems to have considerable promise as an experimental animal, and, after briefly outlining its merits for this purpose, to provide information pertinent to its use in the laboratory.

C. Merits of Household Lepismatids for Experimental Use

The largest of the commonly available apterygotous insects are two species of the family Lepismatidae which occur in human habitations, often in abundance. They are the silverfish, Lepisma saccharina Linn., and the firebrat, Thermobia domestica (Pack.). Although apparently indigenous in Europe and Asia they have been carried by commerce to nearly all parts of the civilized world. Since these insects are seldom serious pests and are easily controlled by the use of poisoned baits they have received no special attention from entomologists and adequate methods for rearing them have not been generally known.

Before discussing these species further a few of the characteristics of the family may be mentioned. The lepismatids, like other Apterygota, have no metamorphosis but molt an indefinite number of times throughout their lives. Thus the interruptions for pupation and wing-formation, which occur in most insects, are absent. Their powers of regeneration are quite remarkable. Their fragility forbids direct handling but they are easily retained and transferred in open glass dishes, for they cannot climb on polished, vertical surfaces; neither can they leap. They are soft of body, easily dissected, and easily prepared for histological sections. They produce hard, dry, fecal pellets and emit no perceptible odor. Their eggs are laid singly, are comparatively large for insects, non-adhesive, and are excellent subjects for embryological study, as shown by Heymons (1897). The known life-spans of lepismatids are rather long and reproduction begins before the animal is fully grown. The reproductive organs and processes are of a remarkably primitive type. Because they are often supposed to be similar in constitution to the hypothetical evolutionary ancestors of winged insects their value in studies of comparative anatomy and physiology should be considerable. The spermatogenesis of the firebrat has been studied by a number of workers and found to be of particular interest (Mukerji, 1929).

The two lepismatids mentioned above as guests in human habitations are quite different in many respects. While the silverfish has the advantage of breeding at ordinary room temperatures it is smaller and swifter in its movements than the firebrat. Those kept by the author have proved difficult to work with owing to their extreme shyness of light and their elusiveness generally. For this reason it is likely that the larger and less excitable firebrat is more practical for laboratory use. This species has been reared by the author for years (Adams, 1933).

One of the most outstanding, special characteristics of the firebrat is its thermophilia. It not only prefers but requires temperatures much above those at which most organisms thrive. In suggesting lines of research which may be carried on with it, therefore, the physiology of high temperature (thermophilia and thermoplegia) must be mentioned first. (A publication upon this topic is to appear elsewhere.) The author has also used this insect in the study of gregarine parasites. (Adams and Travis, 1935.) Tests of the toxicities of arsenic trioxide, sodium fluoride, and thiodiphenylamine were made, again using the firebrat (Snipes, Hutchins, and Adams, in press). This study further suggested its use in gaining data upon the vitamin requirements of insects. Other lines which are suggested are: physiology of tropisms, physiology of water relations on dry diets, biochemistry of insect feces, studies on regeneration of appendages, developmental insect morphology, arthropod phylogeny, animal sociology, and the ecology of crowding. The reasons for these recommendations should become apparent from the data below.

D. Historical upon Rearing Methods for the Firebrat

Firebrats were kept in captivity, and probably reared throughout most of their life-cycle. by Oudemans (1889) in Holland. This observer kept the insects in glass dishes in an embedding oven at 30°C. He provided for humidity by placing in the apparatus a moist sponge. He studied the external parts of individuals of both sexes and various sizes, published a splendid drawing of the insect, and made comments upon the habits. Forty-one years later Spencer (1930) briefly outlined the requirements of the insect without presenting the details of his method (which had been part of an unpublished thesis, 1924). Two years later the author published a preliminary paper upon the biology and cultivation of firebrats (Adams, 1933). The present paper proceeds further upon this topic.

E. The Value of Reared Specimens

The rearing of firebrats in the laboratory in which they are going to be used has at least three important advantages. First, large numbers in uniform series can be obtained; second, the hereditary and environmental histories of the specimens can be accurately told; and third, laboratory rearing affords an opportunity to gain an intimate acquaintance with the peculiar manners of the living animals, invaluable for the recognition of abnormal states and the improvement of cultural and experimental technique.

It is true that the laboratory animal, owing to its exceptional environment, is not entirely representative of its species; as Spencer pointed out (1924), firebrats from the highly favorable conditions of the rearing cabinet have greater size, larger fat-bodies, and slower movements than their "wild" and hungry contemporaries. Furthermore, segregated rearing might lead to the production of racial strains differing genetically from wild stock. For these reasons it is imperative that the experimenter preface his data with a history of the specimens used.

II. METHODS AND OBSERVATIONS

A. The Local Habitat and Source of Laboratory Stock

The depismatids obtained by the author in the vicinity of the Iowa State College during the course of this study, 1931-1935, represented three genera: *Lepisma*, *Ctenolepisma*, and *Thermobia*. The firebrat, *Thermobia domestica*, was the most abundant.

As a result of examinations of many infested college buildings certain generalizations may be presented regarding the local habitats of the firebrat:

1. Firebrats were found in close association with artificial heat. Basement rooms containing numerous hot-water pipes and reservoirs and providing nooks too warm for human comfort were abundantly infested.

2. The heat in these infested rooms was supplied during the entire year almost without cessation.

3. In all examined cases of abundance there was access to one or more of the following: farinaceous meal, heaps of crumpled paper, books, coollen and silk materials, decayed wood, kitchen refuse.

4. In all examined cases of abundance there was access to moisture, such as that of dripping water fix-

tures or moist floors.

5. Partial darkness and comparative freedom from disturbing air currents were characteristic of the favored habitats.

6. Tiny nymphal firebrats were found only in the most warm and favorable rooms of infested buildings. Older individuals seemed to have spread out to the less favorable rooms which were at ordinary temperatures. The writer agrees with Spencer (1924) that this spread is due to hunger. The typically infested building has one very warm nook (over 30°C.) in which the insects breed and from which larger individuals go forth to other rooms on the same and adjoining floors in search of food.

7. The tunnels joining buildings in a centralized heating system have been suggested as conducive to the local dissemination of firebrats. It seems likely that, as a rule, in Iowa, the firebrats do not migrate from building to building except through warmed conduits, and that their spread is otherwise dependent on human agencies of transportation.

B. Methods of Obtaining Firebrats

A good method of capturing firebrats without injuring them employs an ordinary tumbler or wide-mouthed glass jar and a stiff card. The tumbler is inverted upon the insect where it runs on wall or floor. The card is then slid under the mouth of the tumbler and the whole quickly inverted. The insect is then shaken into a jar containing folded paper strips.

Where the insects are difficult to catch traps or pitfalls may be set up in their haunts. The simplest consists of a glass dish such as a large shallow culture dish or even a tumbler with straight, vertical sides, wrapped in a cylinder of paper. The paper should project about an inch above the glass rim. The natural activity of hungry firebrats will lead them into this trap if it is well placed. The effectiveness is improved by placing a narrow ring of wet flour around the rim of the glass. The presence of the food leads to congregation and fighting upon the rim and increases the likelihood of firebrats slipping into the glass.

Since firebrats are evidently unable to sense their food at a distance, but must find it by palpation, it is not surprising that trap catches are not large. Traps are described here merely to aid the beginner in securing living specimens for rearing stock.

C. History of the Stock

In all, about one hundred firebrats were caught in 1931 in the various campus buildings at Iowa State College and placed together in an incubator. From these have been descended all the firebrats used in this study. Regarding the genetical constitution of the firebrats so secured it may be said that all groups used as segregated colonies have come from eggs laid in cages containing many animals of both sexes. The practice of segregating the young of one female, or one pair, and deliberately allowing them to inbreed has been avoided. No obviously freakish animals have been noticed in thousands of individuals. Regarding the environmental background of these firebrats information is provided below in the discussion of the methods with which they were reared.

D. Rearing Methods and Equipment

Firebrats were reared in the cabinets, providing constant temperature and humidity, which were described by Brindley and Richardson (1930).

1. The culture cages.

The type of culture dish to be recommended depends somewhat upon the purpose of the rearing. When hundreds of insects are to be reared in a small space without close attention, open half gallon fruit jars, or aquarium jars, are excellent. Where periodic examination of the specimens is described shallower dishes are advisable.

For general rearing the type shown in Fig. 2 is recommended. It consists of a glass culture dish about 20 cms. in diameter and 8 cms. in height. Paper strips 4 cms. in width and 30 cms. long are plaited transversely, the plaits occurring about every two centimeters so that the folded strip resembles the collapsible side of a bellows. Twenty or more of these folded strips are stood on their edges in the culture dish. A glass cylinder, half-filled with cotton batting, is laid in the dish on one side. Food is placed upon the bottom among the paper strips and if rapid rearing is desired a watering-tube is placed in. This consists of a slender test tube of water plugged with cotton batting and inverted in the dish so that the free surface of cotton remains moist. Such a cage accommodates one to two hundred firebrats.

Where a careful check on the oviposition rate is desired the ovipositing colony may be placed in a similar culture dish containing no paper but with three clay flower pots, graded in size, inverted in the dish, as shown in Fig. 1, so that the largest pot covers the medium one and the medium one covers the smallest. The edges on one side are supported by a thin

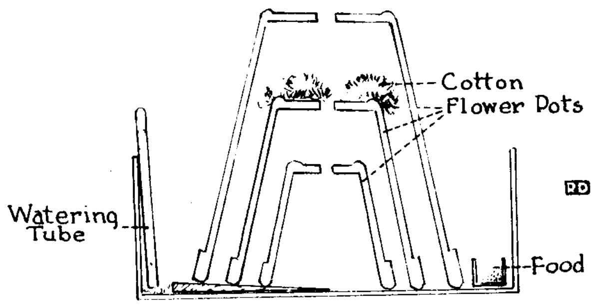

Fig.1

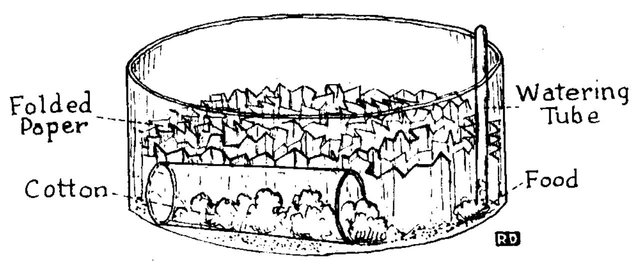

Fig.2

Fig.1. Cross-section of rearing apparatus for the firebrat, for use in ovipositional studies.

Fig.2. Sketch of apparatus for general use in rearing the firebrat. The watering tube and the oviposition-cylinder are usually omitted

wooden wedge. Cotton batting to receive the eggs is placed between the outer and the middle pots. This type of cage is not conservative of space but it is very satisfactory for close checks on oviposition, for nutritional studies, and for the production of perfect specimens. No more than fifty firebrats should be confined in the flower-pot type of cage. Food may be provided in a small paper box.

The eggs of the firebrat are easily removed from the cotton in which they are laid; but, unless it is necessary to carefully count them, it is more convenient to transfer them, in the cotton, to an incubation dish containing paper strips closely packed together and otherwise equipped as a rearing dish. The newly hatched nymphs will live in loose cotton batting if it is dusted with ground oatmeal. But, as the fourth instar approaches, and their first fragile scales are soon to appear, they leave the cotton for the interstices of the paper. It is wise to brush and dry a thin layer of flour paste upon the floor of the dish to provide for tarsal traction.

Where it is necessary to rear nymphs individually or in small segregated groups these conditions may be duplicated on smaller scale in vials. The author has been unable to prevent a high mortality in nymphs reared singly. As Spencer remarked, the young nymphs seem to require proportionately more space than the adults.

Adult firebrats may be kept singly or in pairs in covered Petri culture dishes, 10 cms. in diameter. They are kept in such dishes in studies upon their gregarines, where low-power microscopic examination of the feces is part of the routine.

A number of precautions are to be observed in keeping firebrats in large culture dishes. To prevent escape of the firebrats the glass must be clean, particularly that area of it which is between the top of the papers and the rim. The scales which accumulate from insects trying to escape should be removed. To prevent invasion by book-lice and mites a thin barrier-strip of vaseline should be run around the outside of the dish. Since an excess of food in the bottom of the dish offers a breeding place for these forms and, if moistened, a place for molds also, it is wise to supply only as much food at a time as the insects will eat in a week or two. The watering tube, if used, may be stood in a small paper dish to keep its moisture away from the food, which is likely to be spread about the floor of the cage by the insects.

2. The temperature.

A temperature of 37°C. was used for most of the rearing although 34.5°C. and 38°C. were satisfactory. It was found from data being published elsewhere that the central point of the preferred range for the firebrats used was near 37.5°C. This temperature is therefore recommended. Firebrats may be kept in good condition at high room temperatures, about 28°C., if the humidity is kept up, but they will show little growth or reproduction. Cool conditions are useful in checking firebrat development during temporary suspension of the investigation.

3. The humidity and the use of water.

Firebrats supplied with moist cotton wicks can live in fairly dry air (50 per cent R. H. or less). But in a closed space over saturated magnesium chloride solution, calculated to give a relative humidity near 32 per cent, adult firebrats died in ten days, showing extreme shrinkage of the abdomen. The insect seems to prefer a fairly high humidity. The humidities obtained over brine, containing an excess of the sodium chloride, were found to be satisfactory. A large basin of the brine is exposed under the fan in the constant-temperature cabinet. The resulting relative humidities were found, by psychometric and hygrometric tests, to rise to points between 70 and 75 per cent. Firebrats reared at much lower humidities require watering and at much higher humidities the foods are likely to mold.

Although firebrats show a strong avoiding reaction when their antennae touch open water they are attracted to damp surfaces of paper, cotton, or sponge. The wet cotton plug of the inverted watering tube is avoided but the visibly damp paper surfaces in contact with it are preferred resting places for the insects, and such paper is frequently gnawed by them. Spencer (1924) has observed that females, before oviposition, rested for hours with their abdomens curled against the damp roll of paper projecting from a watering vial. He is of the opinion (1929) that firebrats take moisture through the body wall and keep up their moisture supply by periodic visits to damp surfaces. It is evidently in this manner that they live in the comparatively dry air of warm buildings.

In air at about 75 per cent R. H. firebrats have been reared for generations without watering. There is, however, at this humidity, still some tendency for the insects to cluster about a watering tube and watering has been found to increase the rate of oviposition and growth. It is evident that the optimum relative humidity for the insect may be considerably higher than 75 per cent although the latter is satisfactory for practical purposes.

4. Air circulation.

For the purpose of equalizing air conditions throughout the temperature cabinet, air was kept in gentle motion, perceptible to the hand, across the open tops of the culture dishes. The use of lids upon the culture dishes was necessary only where the entrance of micro-organisms such as gregarine spores was to be avoided. Quick gusts of air, particularly of differing temperature, are so exciting to firebrats that it is probable that static air is most favorable for their well-being. Half-gallon fruit jars without lids seem to provide excellent conditions in this respect.

5. The light.

The adult firebrat is negatively phototropic in strong light but it seems to display little preference between weak light and darkness. In these rearings the insects were exposed to a variable illumination in the twilight range of intensity.

6. The food and nutrition.

The most extensive investigation of the food preferences of *Thermobia* was made by Wakeland and Waters (1931) in searching for a suitable bait. Of a large number of foods, offered in conjunction with dry wheat flour, the insects ate the largest quantity of moist wheat flour. Since, however, it was not practical to use moist food, the preferred dry food, oatmeal, was adopted for use in poison bait. It was found that the insects ate more of this material when sugar and common salt were added. The mixture finally adopted consisted of: oatmeal, finely cut or ground, 100 parts by weight; granulated sugar, 8 parts by weight; common salt, 2.5 parts by weight.

Wakeland and Waters found that firebrats having access to flour ate very little of animal foods such as egg-yellow, dried milk, meat meal, dry glue, dry gelatine, and dry egg-white. Spencer (1924 and 1929) placed ten hungry, freshly caught firebrats in a culture dish in which were two heaps of food, one of flour and the other of pulverized lean veal. At the end of ten minutes nine of the insects were feeding on the meat and only one on the flour. Spencer states that the insects became greatly excited upon contacting the dried meat and fought with their fellows for possession of it. The author is of the opinion that there is no contradiction in these results of Spencer, and Wakeland and Waters; that protein hunger in these insects is sometimes acute, but that it is quickly satisfied.

In rearing the firebrat at Iowa State College rolled white oats (Quaker Oats Co.), lean beef, dried and ground in the laboratory, dried brewer's yeast (Harris Laboratories, Tuckahoe, N.Y.), sucrose, and sodium chloride were the ingredients of a highly satisfactory diet. The five materials were placed in the culture dishes in separate heaps or in small paper boxes. While satisfactory growth was obtained in firebrats reared on rolled oats alone, it was found that additions of beef, sugar, and yeast were markedly beneficial. In an experiment at 38°C., 384 tiny nymphs mostly in fourth instar were distributed in two series consisting of four rearing jars each. The details and the results are presented in Table I and Fig. 3. It will be seen that the gains in weight were smallest, and the decline in numbers greatest, with the unsupplemented rolled oats diet; and that, of the additions, dried brewer's yeast was the most effective.

Table I

Showing the Results of Tests of Various Diets upon the Growth of Firebrats from the 10th to the 96th Day

No. insects at start	Dietary (no water)	Final number of insects	Total weights in mgs.	Average weights in mgs.	Combined average weights in mgs.
48	Rolled oats	17	196.0	11.5	
48	Rolled oats	14	120.0	8.6	10.2
48	Rolled oats, dried beef	27	365.0	13.5	
48	Rolled oats, dried beef	36	396.0	11.0	12.1
48	Rolled oats, dried beef, sucrose	25	339.0	13.5	
48	Rolled oats, dried beef, sucrose	30	371.0	12.4	12.9
48	Rolled oats, dried beef, sucrose, yeast	21	352.5	16.8	
48	Rolled oats, dried beef, sucrose, yeast	28	429.0	15.3	16.0

The results of unsuccessful attempts to rear firebrats on dried beef alone and on paper glaze alone are already stated (Adams 1933).

Rearing experiments have been conducted to determine a basal diet for nutritional studies. The results based on a few hundred specimens reared in small groups are not conclusive. It was found that the insects could be reared from the egg to maturity on a diet consisting of the following (Harris products).

Purified casein, vitamin-free . .	20.5 parts
Purified starch, vitamin-free . .	75.0 parts
Complete salt mixture	4.5 parts
Dried brewer's yeast	5.0 parts

Growth upon this mixture was about the same as that upon rolled oats alone. Growth upon this mixture with the yeast omitted was decidedly inferior to that upon rolled oats, producing no full-grown animals.

As to the proportions of foods consumed, the results of a test are presented: a colony of firebrats was given access to measured quantities of rolled oats, dried lean beef, and granulated sugar. The amounts consumed were 470, 252, and 315 milligrams, respectively.

The firebrat is evidently an omnivorous feeder. Hungry firebrats will eat such varied foods as cheese, hard butter, nut-meats, sliced apple, raisins and wilted lettuce. Fatty

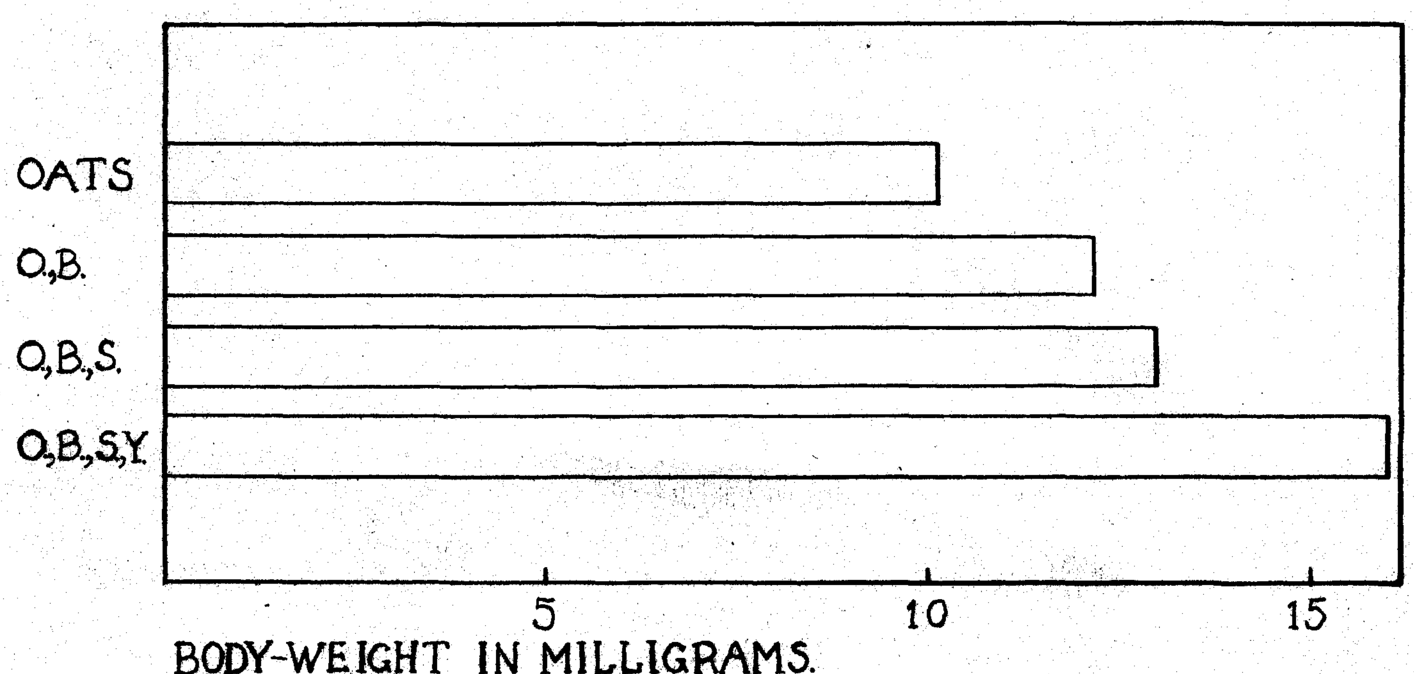

Fig.3. Bar chart of data in last column in Table I, page 26, showing the average weights of firebrats 96 days old, reared on various diets. O., oats; B., beef; S., cane sugar; Y., dried brewer's yeast.

and watery substances in the liquid state, when touched with the antenna, are strongly avoided. In certain types of nutritional studies the insects must be reared singly owing to cannibalism.

E. Notes Upon the Life-history

A detailed life-history of Thermobia domestica closely following the relation of molting to development has still to be worked out. Brief contributions have been made by Oudemans (1889), Spencer (1929, 1930), and Adams (1933a and 1933b). Some additional facts of importance in rearing may be presented here. At 37°C. and other conditions as recommended in this paper firebrats are sexually mature in about three months and reach maximum size and weight in five to six months. The life span extends upward of one and one-half years at this temperature. Figs. 4 and 5 are drawn from ventral views of firebrats of first and mature instars respectively.

A few facts about the instars may be mentioned. In the first three instars firebrats are scaleless. In the third instar the antennae, which are of utmost importance to the insect in food-finding, already exceed the body in length. The fourth instar nymph bears scales and, on the ninth abdominal

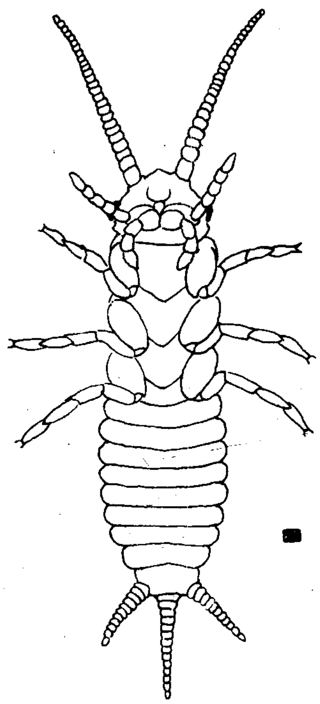

Fig.4

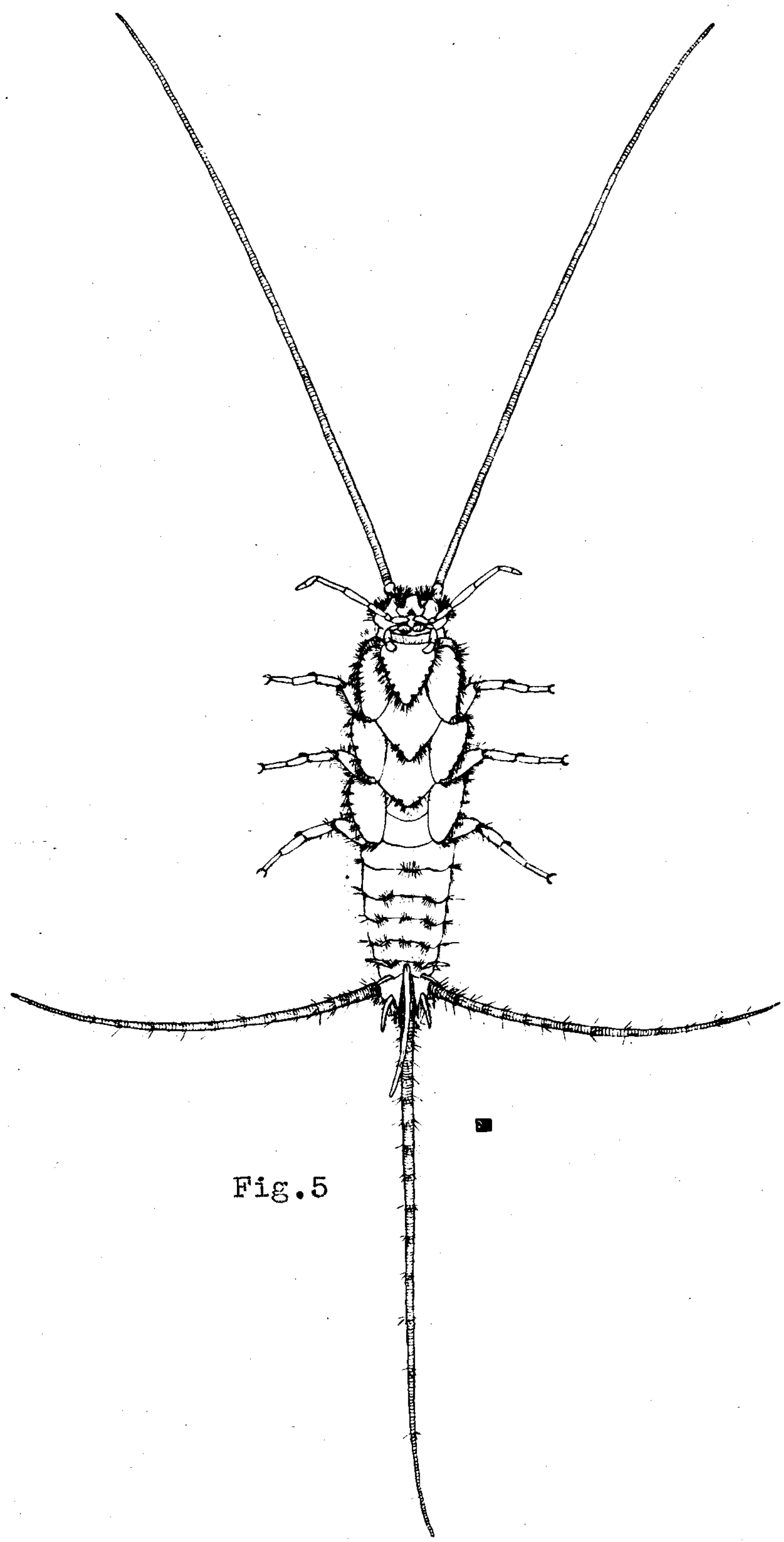

Fig.5

Thermobia domestica, ventral aspect.
Fig.4. First instar (x30). Fig.5. Mature female (x4.6).

segment a pair of short styli. In the fifth instar these styli become elongated and deflexed from the longitudinal axis of the body, and the body scales are variegated so as to form a dark and light pattern. In the eighth instar the sexes can be distinguished by the developing genitalia, and it is at about this time that the second pair of styli appear. It requires two or three more molts to bring the ovipositor to functional length.

While it may be that each instar during growth is definitely characterized by certain steps in structural development, the identity of the instar cannot safely be based on the usual measurements of head-widths, etc. Insects of the same age and instar show greatly increasing individual variation in dimensions as they grow older. Moreover, in this soft-bodied insect, there is considerable growth during the instar period. For example, the average metanotal width of ten nymphs nearly ready to molt out of the third instar was over nine per cent greater than the average for ten nymphs just molted into this instar.

Studies upon development of individual firebrats are rendered difficult by the high mortality in specimens reared in solitude. Moreover, owing to their great activity, the insects have to be rendered inert before any minute examination can be made. One hour in a refrigerator at 2°C. was found effective in rendering young firebrats motionless so that they could be measured with an eye-piece micrometer; although this method, for obvious reasons, can not be used without retarding the development of the subjected animal.

The development and molting between the attainments of sexual maturity and of gravimetric maturity have not been studied. The largest male on record, which had a length of 12 millimeters and weighed 34.5 milligrams, showed only two pairs of abdominal styli, although other males have been seen with three pairs evident. The females are regularly larger and heavier than the males and regularly acquire styli on the seventh abdominal segment before reaching maximum size. The external measurements and weights of firebrats fluctuate remarkably with conditions of nutrition and moisture. In specimens suffering from lack of moisture the abdomen may shrink as much as half normal length before death.

F. Sexual Habits and Oviposition

The sex ratio in this species is evidently one of theoretical equality. The sums of the first nine populations counted were: 308 males and 305 females.

Study of the sexual habits, which requires a great deal of patient observation, has been made by Spencer (1930), who discovered the peculiar method of sperm transfer which replaces copulation in this species, and probably in other lepismatids. In confirmation of Spencer's observations the following statements result from incidental observations of the author. Firebrats have never been seen in copulation, but on the contrary, they have shown a marked aversion (doubtlessly associable with their fragile structure) for contact with each other. The dance, described by Spencer in his unpublished thesis (1924), in which the male deposits on the floor a spermatophore and the female takes it into her genital tract has not been observed to completion, but a peculiar interplay of the antennae between males and females has often been observed. The presence of one to three whitish, spheroidal balls of sperm, about 250µ in diameter, in the spermatheca of the reproducing females has been repeatedly demonstrated. The extrusion from the penis on an etherized male, of a whitish globule which quickly hardened into a spermatophore-like body, is mentioned as further evidence.

It is clear that the presence of males is essential to reproduction. In an experiment five females were reared singly from the egg and placed under conditions at which other females were ovipositing. They attained evident sexual and gravimetric maturity and were observed for nine months; but they produced no eggs in the absence of males.

Another experiment was made to determine the effect upon ovipositing females of removal of males. A colony containing about one hundred and fifty individuals of both sexes was placed under observation at 34.5°C. for about a month. During this time about one thousand eggs were removed in cotton from this colony. A week afterward twenty-two of the females were set aside in a similar environment but without males. The decline in their egg-production is shown by the data obtained on the following days:

4th	day:	50	eggs	found	in	cotton
7th	" :	64	"	"	"	"
13th	" :	1	"	"	"	"
19th	" :	0	"	"	"	"
28th	" :	0	"	"	"	"

All the eggs collected on the fourth, seventh, and thirteenth days hatched in due time. When males were replaced with these females egg production was resumed in a few days.

In another experiment nine females which had produced no eggs during three weeks of segregation were placed with nine males. Six days later 55 eggs were collected from this colony and on the twelfth day, 109 eggs.

In order to determine the interval between the introduction of males and the commencement of oviposition two virgin females about fully grown were placed with two hitherto isolated males in a suitable environment. On the fourth day following, six fresh, white eggs were collected and these, on incubation, proved fertile.

The actions of ovipositing females may be mentioned. Females about to oviposit move excitedly about over the surface of the provided pads of cotton batting; the flexible ovipositor, which is usually carried parallel with the longitudinal axis of the body, is deflexed in a curve till the tip is quite at right angles to the body axis. The highly movable tip becomes engaged in incessant probings of the cotton. The styli are also in motion. One role of the ovipositor seems to be the finding and enlarging of a hole in the cotton mesh for the reception of the egg. When the deposition of the egg occurs the tip of the abdomen is pressed into the cotton. Whether the egg passes from the tip of the ovipositor or from a slit at its base, (through which the spermatophore is believed to be taken in) remains uncertain.

G. Reactions to Disturbance

One of the most sensitive criteria of firebrat response to stimuli is the rate of oviposition. It has been shown that firebrats disturbed daily lay many less eggs than those disturbed only at longer intervals (Adams 1933b). The disturbance in this case included removal of the open culture dish, containing the insects, from the incubator to the laboratory table, the exchange of cotton batting, the addition of food, and replacement in the incubator. In such treatment the firebrats, which remain motionless a great deal under constant conditions, become highly active, particularly if the air currents caused by the investigator's movements, or his breathing, strike them directly.

It is the belief of the author that a factor of prime importance in the distribution and abundance of these insects is freedom from air-current disturbance. The insects are well known to abound in temporarily closed buildings in summer and in warm storage rooms in which there is little human traffic. In rooms such as kitchens, which are much inhabited, the insects are seldom seen by day, although they may be seen there at night after the room has been still and dark for some hours. It is true that the firebrat is decidedly negatively phototropic (although not nearly so much so as is the silverfish). But the responses to light are slow, whereas those to air currents, particularly where a sharp change of temperature is involved, are marked by violent outbursts of running. Where they are protected in their culture dishes by use of the glass lids firebrats will carry on feeding, fighting, and ovipositing in the normal daylight upon the laboratory table. Spencer (1924), who has made most careful observations, states that the nymphs are highly negatively phototropic in the early instars but later begin to wander out into the light in search of food. He associates the appearance of the insects in day-lighted rooms with hunger. He is in agreement that the avoiding response to air-currents is of major importance in the insect's system of self-protection.

H. Social Habits

The only sign of gregariousness noted in this insect is a tendency, remarked both by Spencer and the author, for the resting insects to remain in a loose group in a certain part of their cage in spite of an apparent uniformity of conditions over a wider area. A fully grown firebrat, with a body 12 mm. in length, antennae 18 mm., cerci 12 mm., and median caudal filiament 15 mm.; is in need of more than eleven square centimeters of area in which to disport its parts. The appendages, though readily replaced in molting, are fragile and frequently broken; the scales and bristles of the body are easily damaged. When placed in a glass dish without paper to climb upon the insects run incessantly round and round the confining wall and, milling over one another with their clawed feet, they quickly erode most of their scaly coverings. In view of such fragility it is not surprising that a firebrat usually shows avoiding responses whenever it is touched by another of its kind. To this only sexual activity and fighting are exceptions.

Fighting is a common occurrence between hungry firebrats and takes two forms. The first is shown when the insect is feeding upon a morsel of food for which another competes. The two insects coming face to face elevate the fore parts and seem to clash with antennae, jaws, palpi and forelegs. In some cases this seems to be little more than the attempt of one insect to snatch food from the jaws of another. Such encounters are of only a few seconds duration. The second type of pugnacious activity is shown by a firebrat which becomes surrounded by its fellows in feeding. The abdomen is twitched violently from side to side causing a rapid and effective scythe-like motion of the lateral cerci and caudal filiaments. Well fed firebrats are not strongly cannibalistic. The bodies of injured individuals are quickly eaten, but healthy adult individuals are able to live together even under fairly crowded conditions without showing much mortality which can be attributed to this cause. Under pressure of starvation the exuviae are almost entirely consumed. Spencer states, however, that firebrats are sometimes devoured alive while molting. It is true that in these experiments, among firebrat populations of all ages, there has been considerable mortality, the primary causes of which have not yet been adequately studied.

III. DISCUSSION

The material contained in this paper is strictly introductory and exploratory in character. It is evident that none of the rather miscellaneous topics have been given intensive study here. Each has been opened up as a source of suggestions for further research, in the belief that lepismatids have a great potential value as laboratory animals for various biological studies.

The future worker is warned that an air-conditioned incubator is practically indispensible in the rearing of this insect, and that its fragility and elusiveness might be decidedly disadvantageous in some studies. It may be, however, that the necessitation of the incubator is a blessing in disguise, lending greater exactness to the preparation of living material, particularly for delicate experiments.

It is hoped that this paper will not only stimulate interest in the firebrat but also in other lepismatids. A similar technique for rearing Lepisma saccharina L. should be worked out. This insect according to Sweetman (1934) can be reared at 28°C. and 90 per cent R. H. There is also the brown bristle-tail, Ctenolepisma, which is sometimes available in buildings and seems to require conditions similar to those for the silverfish.

IV. CONCLUSIONS

1. Firebrats may be reared with ease in the laboratory by the fulfilment of conditions stated in this paper.

2. The firebrat has been used successfully in experimental studies and has considerable promise for this purpose along a number of lines of research.

3. In two respects the firebrat is highly unusual: in its thermophilia and in its method of insemination (although it is probable that the latter occurs in other Thysanura).

Further and detailed conclusions, in a paper which chiefly reports methods, would seem superfluous.

V. SUMMARY

The primitively wingless insects (Apterygota) have been very little used as experimental animals. The use of the common, household, lepismatids, the silverfish and the firebrat, is suggested. The firebrat is the larger and more tractable of the two, and has the remarkable characteristic of being very thermophilic. Although fragile and slow of development, firebrats have proved excellent subjects for studies upon lepismatid biology, insect toxicology, thermophilia and thermoplegia, and gregarine parasites. They are suggested for studies upon regeneration, embryology, gametogenesis, insect nutrition, and animal sociology.

At Iowa State College firebrats are found associated with the heating system which provides nooks in the buildings and tunnels where temperatures above 30°C. prevail most of the year. From about one hundred captured specimens the author has reared many generations, including thousands of descendants, over a four-year period. The insects are reared in glass culture dishes containing plaited strips of paper, at 37°C. and a relative humidity of about 75%, in moving air, and in semi-darkness. They are fed upon rolled oats, dried lean beef, dried brewer's yeast, cane sugar and common salt, each material being supplied separately. They can also be reared, but more slowly, upon rolled oats alone or upon a

basal diet of starch, casein, and "complete salt mixture", supplemented with dried yeast. Under favorable conditions firebrats reach maximum weight in about five months. Individuals of the same age show increasing variation in their sizes as they pass beyond the early instars. There is evidence that such structures as the metanotum grow as much as nine per cent in width during the third instar. The sexes cannot be distinguished without minute examination until the eighth instar. The males and females are about equal in number. With regard to the sexual habits the writer agrees with Spencer that copulation is absent and that the females take up spermatophores dropped by the males. Balls of sperm have been found in the genital tracts of females. There is no oviposition in colonies from which males are absent and oviposition ceases in a few days following the removal of males. When hungry, firebrats are fairly tolerant of moderate light. When protected by glass from air-currents (which excite them strongly) they feed and oviposit in the normal daylight of the laboratory table. There is evidence that these insects are somewhat gregarious, although they usually avoid direct contacts with each other. Fighting and cannibalism occur under stress of hunger.

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PART TWO

THE TEMPERATURE RELATIONS OF THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD)(THYSANURA)

PART TWO

THE TEMPERATURE RELATIONS OF THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD)(THYSANURA)

I. INTRODUCTION

The firebrat has long been recognized both in America and in Europe as an exceptionally heat-loving insect. This has been evident in its various names. Packard, in describing the species in 1873, named it *Lepisma domestica* in view of its occurrence about hearths in Massachusetts. In Amsterdam, Oudemans (1889) found the bakers calling insects of this kind "Ovenvogeltjes" (little oven-birds). He used the suggestive name which had been given to European firebrats by Rovelli a few years before, *Thermophila furnorum*. This excellent generic name was found to be preoccupied and the name Thermobia was proposed by Bergroth in 1890. McLachlan (1894) found firebrats in the bakeshops in London; these he called *Thermobia furnorum* (Rov.). Escherich (1905) united the European and American forms under the name *Thermobia domestica*. Spencer (1930), after observing firebrats in their habitats in Canada, stated that they live at temperatures from 90 to 110°Fahr. (equivalent to 32 to 43°Cent.) In his unpublished thesis (1924) he showed that they require temperatures above 30°C. in order to thrive. Adams

(1933), after noting Spencer's work and the habitats of the insects in buildings in Iowa, reared them in great numbers in incubators operated at 37°C. The present account deals in more detail with the high, medium, and low temperature relations.

II. SOME USUAL TEMPERATURE RELATIONS OF INSECTS WITH SPECIAL REFERENCE TO THE CORN BORER

The temperature relations of insects have received a great deal of study which has resulted in some tentative generalizations. After examining the works of many authors, notably those of Chapman (1931, p.34-121), of Pierce (1916), of Sanderson and Peairs (1913), and of Sanderson (1910), who summarized the works of Bachmetjew (1901 and 1907), certain general, or usual, features of the insect-temperature relations may be noted.

First: at favorable humidities many insects, especially those of temperate zones, have their most favorable temperature at a point near 26°C. This point is usually a little above the center of the range of temperatures at which the insect is active - the so-called effective range. Beyond this range, and beginning at highly varied points, are ranges in which the insect becomes dormant due to cold or to heat. Dormancy, or immobility, due to heat commonly begins at about 38°C.; dormancy due to cold commonly begins at points between 15°C. and 1°C. Beyond the temperatures at which more or less protracted dormancy may occur are points at which even short exposures are fatal, probably due to irreversible changes in protoplasm itself. While no general value can be fixed for the low fatal temperature the high fatal temperature, or thermal death point, is commonly found to be very close to 48°C.

Before proceeding with the discussion of the temperature relations of the firebrat, which are quite unusual, and in order to exemplify the temperature relations of insects not particularly thermophilous, some results of a study upon the European corn borer Pyrausta nubilalis Hubn. will be cited.

This study (Adams, 1930) was begun by the author while assistant at the Dominion Entomological Laboratory at Chatham, Ontario, Canada. It had been observed that during May and June there was fairly high mortality among larval and pupal corn borers in the corn litter lying upon the grain-fields. Some of this mortality was attributed to over-heating on bright, hot days. Some workers in Ohio had found a temperature of 120°F. between the stalk and sheath in corn rubbish lying on the ground. (Huber, L. and others, 1928, p.184). To find out if such a temperature could have killed any enclosed insects this laboratory study was undertaken. Full-grown over-wintered larvae were used. It was found that temporary immobility was produced in twelve minutes immersion in water at 112°F. and in forty seconds at 118°F. Larvae were thereafter exposed to high temperatures on a cheesecloth surface, in moving air of controlled humidity, in a specially devised chamber. The results of one test are given in detail. Ten larvae were exposed for one hour to slowly moving air of low humidity (bubbled through a tall cylinder of sulfuric acid of specific gravity 1.55) at

115°F. The larvae went into rapid activity followed by a state of immobility in which there was no response to probing. They recovered at room temperature as shown in Table I.

Table I.

Showing the Recovery of Corn Borer Larvae from Heat Effects

Hours after exposure	Wholly inert	Partially paralyzed	Spinning silk
0	7	3	
8	5	5	
26	4	6	
72	0	8	2
96		6	4
120		1	9
1 week		1	9

A similar test was run using moisture-saturated air at the same temperature. Four days after the exposure eight had regained apparently normal activity, one was partially paralyzed and one was dead. At 118°F. one hour in moisture-saturated air, and two and one-quarter hours in dry air, (35 per cent R.H.) were fatal to all larvae exposed. An exposure of forty-five minutes at 118°F. in air at about 35 per cent R.H. was not fatal; at 121°F. seven out of ten were killed in very dry air and the three survivors never recovered the power of locomotion; and in moist air all were killed. At 125°F. in dry air fifteen minutes was fatal to all larvae exposed.

The greater effectiveness of moist air in short exposures to lethal temperatures was explained in two ways: it has greater thermal conductivity than dry air, and it prevents the insect cooling itself by evaporation of body moisture.

From the above it was concluded that temperatures over 118°F. (about 48°C.) persisting for over two hours or occurring repeatedly in corn stalks might be an important factor in the mortality of contained larvae. Since, however, the insects in the field have an opportunity to raise their heat-tolerance as summer approaches, a need for careful experiments under field conditions to further investigate the rôle of high temperature in the natural control of the corn borer was recognized.

The favorable temperatures for the corn borer have been regarded by Huber and others (1928) as centering around 27°C. The low temperature relations are such as to permit the insect to winter over in the corn litter in the fields in Ontario where the air temperature goes well below the Fahrenheit zero, -17.8°C. Many of the larvae used in the high temperature experiments outlined above had been kept in an exposed place out-of-doors in small, paper-filled, wire-screen cages during February; they revived quickly in the warmth of the laboratory without any sign of mortality which could be attributed to cold. The low thermal death point for these corn borers was not determined owing to lack of apparatus sufficiently refrigerant.

In the course of studies carried on at Biscotasing, Canada, upon a jack-pine sawfly, Neodiprion sps. (Tenthredinidae), the author made observations on the effects of low night temperatures upon broods of mature larvae clustered on pine twigs. The larvae were observed to feed fairly actively at 12°C., slowly at 9°C., and very little at 8°C. But the characteristic responses to disturbance, the elevation of the head and thorax and the extrusion of a sticky drop at the mouth, was performed, although with extreme slowness, at 6.5°C. These larvae retained their positions upon the pine needles even in freezing temperatures; but 6°C. may be regarded as their threshold of visible activity.

III. DEFINITIONS

As Chapman has shown the interaction of factors of temperature and moisture, together with the effects of daily and seasonal fluctuations in these factors, has rendered the ecology of outdoor insects highly complex. Many terms have come into use such as "effective temperature", "absolute minimum temperature", "optimum temperature", and "threshold of development", which seem to be rather ill-defined. No attempt is made here to define terms to be used with insects in general, but certain terms seem very commendable for use in discussing the temperature relations of insects to be reared in the laboratory, under controlled conditions.

1. The Fatal Maximum Temperature: the high temperature of air immediately about the insect, which, under conditions otherwise most favorable, will cause its death by an exposure of just one hour. This is practically the same as the "absolute maximum" or "ultramaximum" of various authors but bears a definite time value.

2. The Maximum Activative Temperature: the highest constant temperature of air immediately about an insect which, under conditions otherwise most favorable, will just permit the insect to retain its power of locomotion, or equivalent strong response, after four hours of exposure to the temperature. This

term is new both in character and definition.

3. The Maximum Completive Temperature: the highest constant temperature of air immediately about an insect which, under conditions otherwise most favorable, will just permit the insect to complete its life-cycle, without regard to the time factor.

4. The General Optimum Temperature: the temperature of air immediately about an insect which, under conditions otherwise most favorable, will itself be "generally most favorable" to the biological success of the insect and its progeny. The quoted phrase comes from Chapman (1931, p.43). It should be noted that this optimum is not to be confused with the so-called optima at which various physiologic processes, or the life-cycle itself, have greatest velocity. As Hoskins and Craig (1935, p.537), and others, have pointed out, maximum rate of growth is not "necessarily associated with maximum vigor and reproductivity". Sanderson and Peairs (1913, p.11) have defined the optimum as "the temperature at which the greatest number of insects will complete their stage, regardless of the time factor".

5. The Minimum Completive Temperature: the lowest constant temperature of air immediately about an insect which, under conditions otherwise most favorable, will just permit the

insect to complete its life-cycle, without regard to the time factor.

6. The Minimum Activative Temperature: the lowest temperature of air immediately about an insect which, under conditions otherwise most favorable, will just permit the insect to retain the power of locomotion, or equivalent strong response, after four hours of exposure to that temperature.

7. The Fatal Minimum Temperature: the low temperature of air immediately about an insect which, under conditions otherwise most favorable, will cause its death by an exposure of just one hour.

Numbers 2 and 3, and 5 and 6, are not to be confused with the maximum and minimum "effective temperatures" of various authors. A distinction of activative and completive minimums is based upon the observation that insects are commonly able to perform locomotion within a range of temperatures much wider than the range within which they can reproduce and complete the life-cycle. The use of constant temperature suggests the importance of this distinction.

The general optimum temperature as here defined would be calculated from the entire vital statistics of the individual or group, and generations of offspring. Such a point might in practice prove quite tedious to determine. The experimenter with a strange insect often wishes to know at once what temperature he should use in rearing it. A ready index to the optimum may be sought by means of experiments upon the thermotactic responses of the individual specimens. The temperature so indicated as preferred by the insects themselves may be termed the preferred temperature, or thermotactic optimum.

8. The Thermotactic Optimum Temperature: the temperature which an insect is most likely to choose by thermotactic responses when given access to a wide range of suitable activative environments which differ only in temperature; or, for a group of insects, the mean point of significantly numerous frequency distributions of the animals, resulting from their thermotactic responses in a suitable thermogradient. (In skewed distributions the modal point might be a preferable criterion.)

The thermotactic optimum or most preferred temperature for the firebrat as determined by the thermogradient studies below has proved to be a highly satisfactory temperature at which to rear the insect (Adams, 1933). It is probable that with such poikilothermic animals as insects the average of the various thermotactic optima obtained with differing individuals and stages is never far different, as a point upon the temperature scale, from the general optimum.

IV. EXPERIMENTS UPON THE FIREBRAT

A. The Preferred Temperature

In order to single out temperature as a factor, to determine the preferred temperatures accurately, and to measure the range of toleration, a thermogradient apparatus suited to the peculiarities of the firebrat was constructed. Its final form is represented in Fig.1. It is plainly a rather crude device, part of its merit lying in its inexpensive construction. It consists of a tin-plated sheet-metal trough 48 inches long, 5 inches high, and 8 inches wide. It rests upon 20 galvanized iron sheets two inches wider and several inches longer than the trough, held tightly together with screw-clamps. The whole is encased in a wooden box lined with asbestos and fibre-board insulation. A 75-watt light bulb for continual heating is mounted inside the box at one end and a copper coil for water-cooling when the room is too warm is arranged in the other end. The trough in which the insects are placed has vertical sliding metal partitions dividing it into twelve chambers. Resting transversely on upper edges of the trough between the sliding partitions are glass lids with fibre-board covers. Each chamber is furnished with a small beaker containing brine with excess of salt (a device to greatly reduce the humidity gradient) and a corked flask of water in which to read the temperature. A

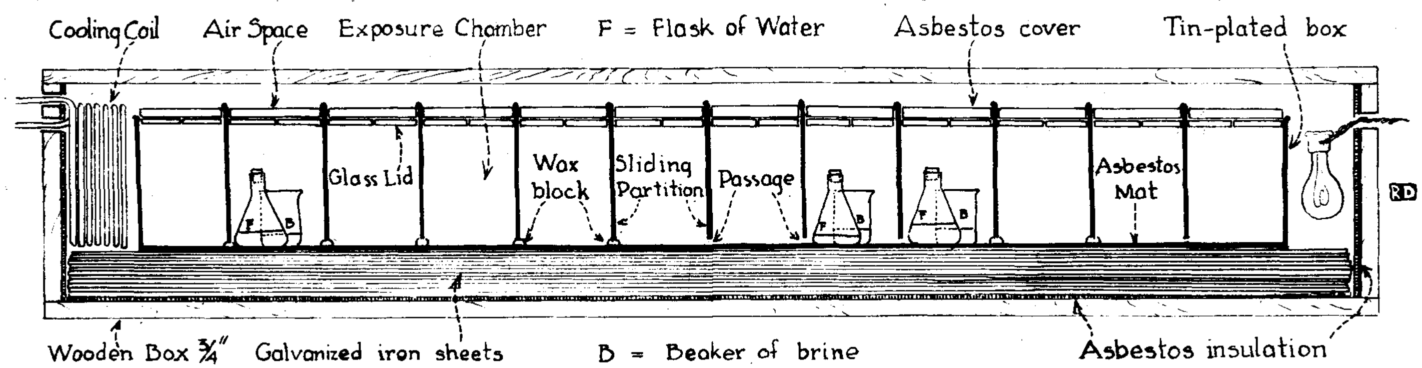

Fig.1. Longitudinal section of thermogradient adapted for determining the thermotactic responses of the firebrat, *Thermobia domestica*.

short thermometer lies upon the floor of one of the chambers to check the reading therein. Rolled oats are strewn upon the thin, asbestos mat which covers the floor of the trough.

In operation the apparatus is allowed to heat for about a day before the insects are put in. The sliding partitions are set with their lower edges resting upon small blocks of wax which hold them about one-eighth inch from the asbestos mat leaving slits through which the insects can pass freely from chamber to chamber. The number of animals used in the gradient at one time varied from twenty-three to forty-six. It will be seen that the apparatus is designed to provide living conditions for the insects over periods of days or weeks. The floor area of any one chamber, 32 square inches, is enough to accommodate nearly all the insects without crowding. It should be noted that the insects are unable to leave the asbestos mat since they cannot climb on the polished metal walls of this apparatus. Before the readings are taken the vertical, sliding partitions are pressed quickly down through the supporting cubes of wax. The insects are thus locked in the chambers in which they have come to rest. The chambers are then uncovered and the temperature of the water in the flask in each chamber, and the number of insects in each chamber, are recorded.

In accordance with the laws of heat conductance the fall in temperature from chamber to chamber is greatest at the heated end and decreases outward in a manner which can be graphically expressed by a decay curve. To secure an even drop in temperature from chamber to chamber would necessitate costly apparatus with separate thermostatic controls in the chambers. With the present inexpensive device the temperature of a given chamber was not constant from day to day. The readings were made after intervals of varying length. The normal activity of the firebrat was depended upon to secure distribution. The insects were obtained from a rearing cabinet operated at 37°C. It cannot be denied that some acclimatization may have been involved, although they did not shun an even higher temperature.

In this series sixteen satisfactory trials were run. Five of them are given as examples in Table II. The Roman numerals refer to the numbers of the chambers. The sum of the sixteen trials is given in the third column of Table III. Each half-degree reading has been combined arbitrarily with the next higher whole degree; this shift serves to offset the tendency of hasty thermometer readings to be a fraction of a degree low.

Two other series of trials were run some months previous to the above, using different specimens. In these the gradient trough was not confined within a box and an electric coil was used underneath the galvanized sheets, which were 18 inches wide and 60 inches long, 15 in number, insulated with strips of asbestos and fibreboard. The inside of the trough was the same

TABLE II

Showing Five Examples of Firebrat Distribution
in a Thermogradient

Temperature Cent.					
47.0	0 IV				0 IV
46.5					
46.0					
45.5				0 V	
45.0					
44.5		1 V			
44.0					
43.5			1 V		
43.0	1				
42.5					3
42.0					
41.5				5	
41.0		2			
40.5			3		
39.5	12			12	10
39.0					
38.5		3			
38.0			10		
37.5				12	
37.0	6				5
36.5		14			
36.0			14	8	
35.5	10				
35.0		11			9
34.5				3	
34.0	9		9		
33.5		9			7
33.0				0	
32.5	3		5		
32.0					
31.5		1			4
31.0				0 XII	
30.5	0		0		
30.0					
29.5					1
29.0					
28.5		1 XII	0 XII		
28.0					
27.0	0 XII				0 XII
No. of firebrats	41	42	42	40	39
Hours between readings	3	4	3	3	9

Legend: The numerals IV, V, and XII refer to particular chambers in the gradient.

TABLE III

Showing the Sums of Distributions of Firebrats in a Thermogradient

Temperature Cent.	Sum of 12 trials	Sum of 6 trials	Sum of 16 trials	Sum of sums
46	1	1	1	3
45	2	2	1	5
44	0	2	1	3
43	19	1	6	26
42	40	7	24	71
41	14	4	18	36
40	55	15	62	132
39	145	24	48	217
38	21	43	65	129
37	30	33	73	136
36	87	6	82	175
35	12	12	30	54
34	22	12	60	94
33	16	9	23	48
32	17	-	11	28
31	2	3	4	9
30	1	3	2	6
29	0	1	1	2
28		1	0	1
Number of Insects:	44 to 38	26 to 24	42 to 23	1175

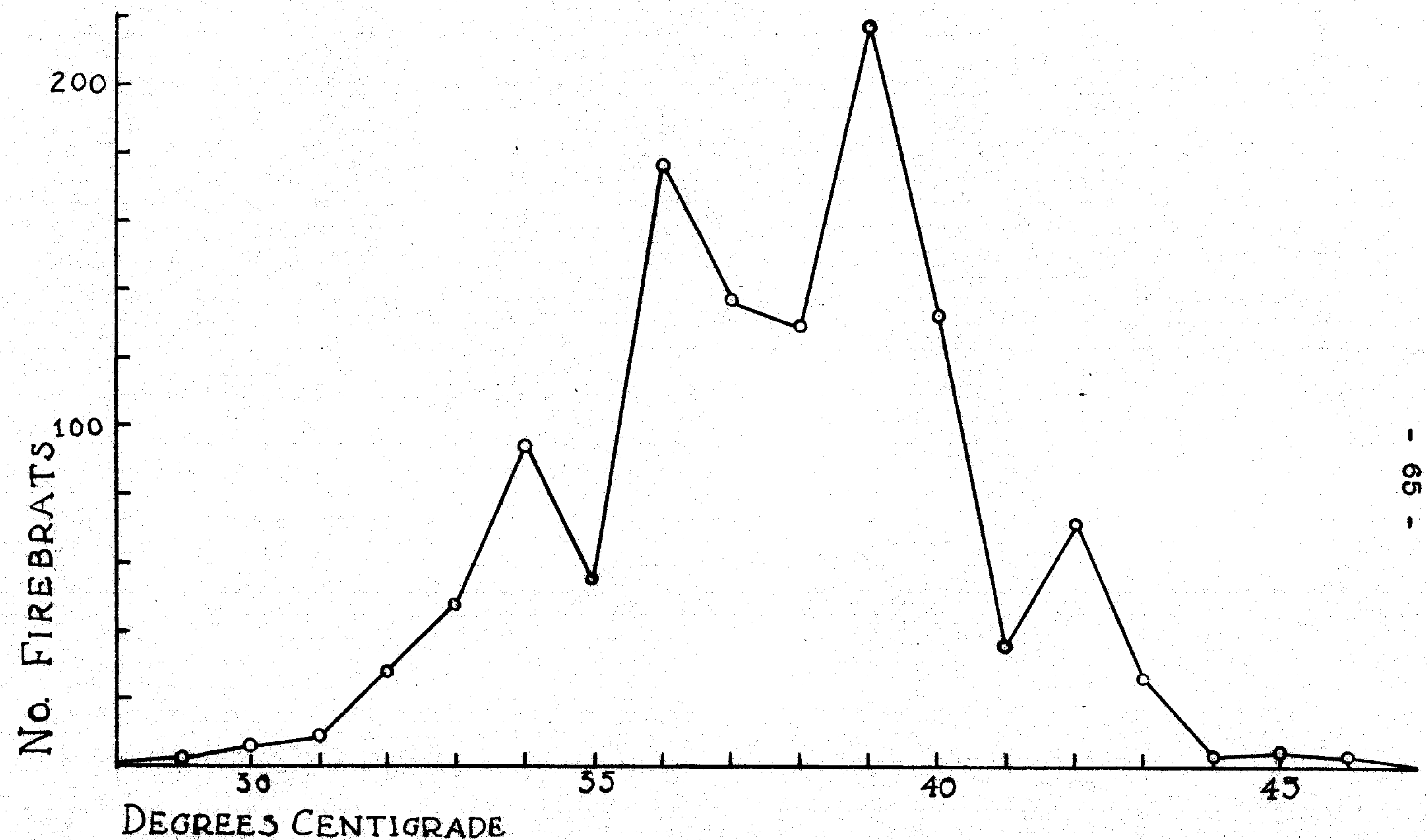

Fig.2. Graph of data in last column of Table II, page 64, showing the sums of distributions of firebrats in a thermogradient.

except that the flasks of water were omitted and temperatures read in air on thermometers thrust through holes in the sides of the trough until they touched asbestos mat. A slender, cotton-plugged tube of water for the insects stood in each chamber. Temperatures from 25 to 55°C. were obtained. The results are summed in the first and second columns of Table III. The fourth column is a sum of the three series of trials giving the distribution of 1175 positions taken by firebrats in the gradient.

It is not claimed that this is an entirely free distribution since only twelve points in the 27 to 47°C. range were read at any one time. And there was no systematic, and probably not enough accidental, shifting of temperatures along the gradient to give each point of the temperature scale an equal number of records. Moreover, the insects were observed to lag in responding to these shifts in temperature, especially near the centre of the range.

The calculated arithmetic mean of the data is at 37.58°C. The larger number of insects recorded at 36 and 39°C. may be accounted for when it is pointed out that the firebrats after being placed in the centre of the gradient move about for some time, many reaching the extreme ends before returning toward the centre. It seems reasonable that these insects returning from the unfavorable ends of the gradient would come to rest a little before reaching the exact centre of the preferred range.

It is evident that the insects were at greatest ease in temperatures at and between 36 and 39°C.

B. The High-temperature Relations

It was interesting to find in preliminary trials that the firebrat adults as well as the corn borer larvae were in accord with the generalization that insects usually have their fatal maximum temperature near 48°C. See Table V. In the tests upon firebrat adults (Adams, 1933) the insects were placed, in lots of four, in an open 50 c.c. flask which was enclosed within a stoppered glass jar that contained saturated brine to stabilize the humidity. The thermometer bulb was in contact with plaited bits of paper upon which the insects rested. The whole was placed in a thermostatic oven. The surprising feature was the ability of the firebrats to endure ten hours at 47°C. without paralysis or dormancy, and a temperature of 45°C. for days without apparent injury. As in the corn borer, the survival was better in dry air than in moist.

In subsequent experiments two colonies of about fifteen firebrats each which had been laying abundant eggs at 37°C. stopped oviposition in a day or two when moved to incubators at 42 and 45°C. When continued at these temperatures, those at 42°C. eventually resumed oviposition while at that temperature those at 45°C. did not. On being returned to 37°C., they resumed their egg-laying in a few days. A few firebrats reared at 42°C. laid some eggs at that temperature; but firebrats accustomed to ovipositing at 42°C., when moved to 45°C. discontinued oviposition and did not resume it at this temperature. The humidities were those over saturated brine.

The effect of high temperature upon hatching time was studied. At 42°C. eggs hatched in as few as 9 days. (Fig.3.) At 44.5°C. and the humidity over saturated brine, of 200 eggs 188 hatched in 10 days, 8 hatched in a few days more and 4 died. Successful rearing from egg to egg was obtained at 42°C. in seven weeks, at which time the forty-eight animals so reared had a total weight of 410.6 milligrams. They were reared from sixty-six eggs. A few attempts at rearing at 45 to 46°C. were unsuccessful, only a few individuals surviving the early instars.

An investigation of the fatal maxima for firebrats in the second instar was conducted. The nymphs were placed in groups of 10 in stoppered test tubes and lowered into a circulating, thermostatically controlled water-bath. Temperature was read on a thermometer thrust through the stopper almost to the bottom of the tube, where the insects clung on strips of paper. Some of the results are presented in Table IV.

Table IV

Showing Results of Exposing Firebrats in Second Instar in Lots of Ten to High Temperatures

Exposure time	°C.	Condition of Removal	Condition two days later
2 hrs.	46.5-46.8	Apparently normal	9 Normal, 1 dead
18 hrs.	46.4-47.0	1 Active, one partly paralyzed, 8 inert	All dead
40 mins.	47.8-47.9	0 Active, 3 partly paralyzed, 7 inert	1 Alive, 9 dead
165 mins.	47.8-48.2	All inert	All dead
360 mins.*	47.8-48.2	All inert	All dead

*Three groups of ten animals were used.

While all the mortality here recorded may not have been due to heat it seems evident that the fatal maximum temperature for these second instar firebrats was around 47 to 48°C.

C. The Low-temperature Relations

Some scattered data based upon preliminary tests are presented here to indicate the general character of the low-temperature relations.

When a colony of firebrats including about thirty females was moved, with only slight changes in humidity, from a temperature of 34.5°C., at which oviposition was abundant, to 29.5°C. the egg production, after a brief pause, continued at about one-fifth the former rate. When, after two weeks at this temperature, the colony was put at 24.5°C. the egg-laying stopped entirely in a few days and did not recommence. In this and in other experiments it is shown that although feeding and the production of gregarine cysts continue to some extent at this temperature, oviposition is stopped. Oviposition is resumed after the insects are returned to suitable temperatures.

The minimum incubation period of eggs, which is thirteen days at the preferred temperature, was prolonged to two months at 24.5°C. Not only was there a high mortality in the young at this temperature but also the growth was extremely slow. Firebrats mature in three months at their preferred temperature but nymphs hatched at 24.5°C. were scarcely twice their hatching length in the same period of time. The minimum incubation periods on record are given in the accompanying graph (Fig.3). The results gave a surprisingly smooth curve. Such a curve might not be obtained if average hatching time were graphed.

Simple tests with firebrats in flasks immersed in running water showed that they became wholly inert at 12°C. Nymphs in early instars exposed for two hours at 12 to 15°C. became inert but returned to activity after a few minutes on a glass surface at room temperature. Adults and nymphs were extremely sluggish

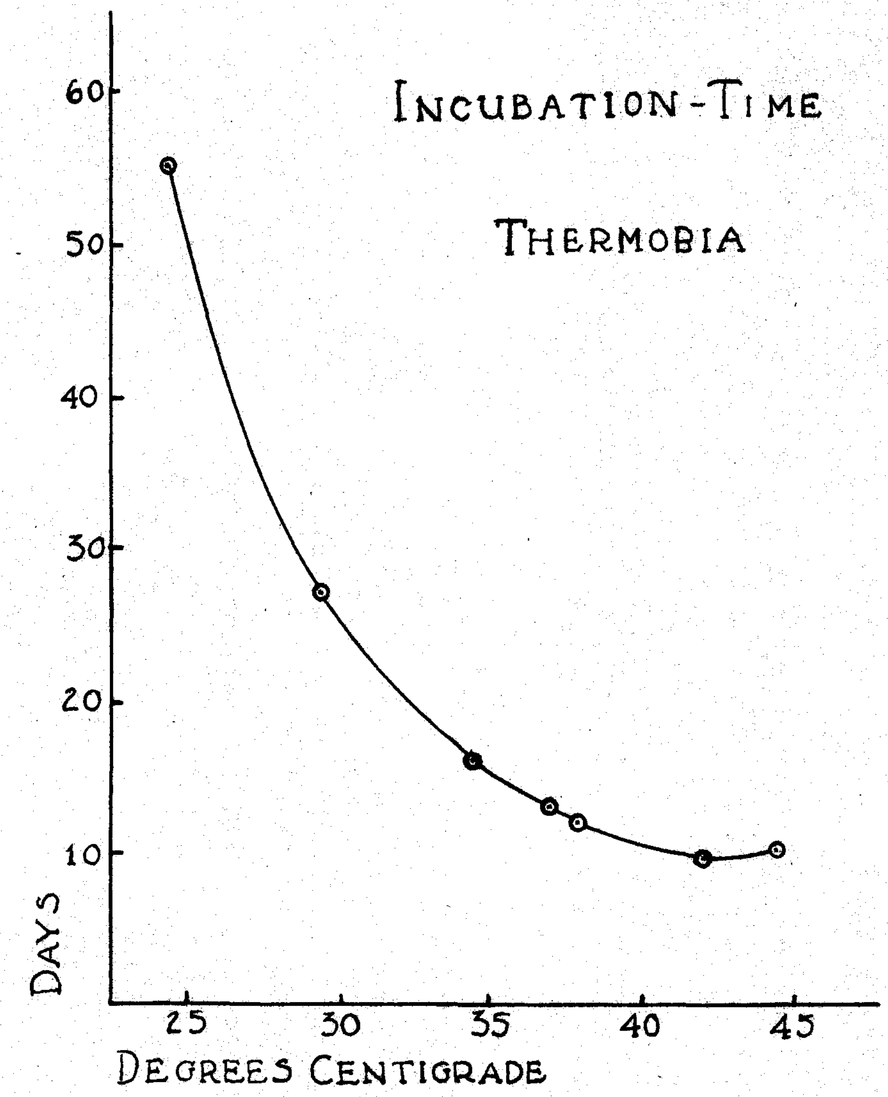

Fig. 3. Graph of the minimum observed incubation periods for firebrat eggs at various temperatures.

between 15 and 20°C. The number of individuals used in these and following tests varied from five to fifteen.

Early instar nymphs and adult firebrats placed in open, thin glass dishes in a refrigerator at 2°C. for four hours recovered, with few exceptions, normal activity within a few days at 37°C. Eighteen hours at this temperature was fatal to half the adults and most of the nymphs exposed. The early nymphs showed decidedly greater cold-resistance than the adults in most of the tests.

Eggs freshly laid at 37°C. and exposed for twenty-four hours at 2°C. hatched with only a small mortality. Only two in fifteen hatched after a two-hour exposure at 2°C. on the third day of incubation. No eggs hatched after exposure to this temperature for ten days. Under the preferred conditions firebrat eggs are almost invariably viable. The controls in these tests hatched one hundred per cent.

A series of tests was run in which firebrats were exposed to moving air at -6 to -10°C. While there were survivors from the shorter exposures, a one-hour exposure at -7°C. was sufficient to kill all the adults and most of the nymphs exposed. A five-hour exposure at -10°C. gave a one-hundred per cent kill. It should not be overlooked that these firebrats were given little opportunity to prepare physiologically for cold. The transfer from the incubator at 37°C. to these extreme temperatures was interrupted by only a few hours at room temperature. Two attempts to bring the insects alive through winter temperatures in closed, capacious jars in a screen-house, by allowing them to begin their exposure gradually in warm, autumn weather, were wholly unsuccessful.

V. DISCUSSION

A tabulation of the gist of this paper is presented in Table V.

The experiments described in this paper show a lack of coordinated planning. This has been due to the fact that they were incidental to other investigations upon firebrat biology, and were often disconnected in time and in purpose. This paper is intended to bring together these miscellaneous results to form a preliminary picture of the relations of temperatures to firebrat life; and, further, to introduce some theoretical and practical aspects suggested by the author's experiences with corn borers, sawflies, and firebrats.

The ability of the firebrat to live actively at temperatures at which most poikilothermic animals are either killed or rendered comatose and the rather abrupt appearance of a critical point near 48°C. indicates that intensive study of this insect might throw light upon the whole topic of high temperature tolerance and the nature of death by high temperature. It is curious that the thermotactic optimum for the firebrat should be so close to the normal temperatures of the human body.

The close link between the distribution of the firebrat and fairly continuous artificial heating is understood when one considers that it does not thrive at temperatures below 30°C. (86°F.) and that freezing temperatures for more than a few hours

Table V

A Scheme of Terminology for Insect-temperature Relationships, With a Comparison of Critical Points of Temperature for *Thermobia domestica* With Those Suggested as Usual for Insects in General.

Zone of temperature	Critical point	Condition of animal	General values for insects	Values for Thermobia
Fatal				
	Fatal maximum	Death in 1 hour	48°C.	48-49°
High inactivative		Dormancy due to: heat		
	Max. Activative		Ca38°(35-45°)	47°
		Metabolism too rapid		
Activative temperatures	General optimum		26°	37°
		Metabolism too slow		
	Min. Activative		Ca8°(1-15°)	Ca15°
Low inactivative		Dormancy due to: cold		
	Fatal minimum	Death in 1 hour	Highly variable	Ca-8°
Fatal				

are fatal. It is evident that low temperature might have practical use in combatting the insect as a pest. Leaving a building unheated for a few days in freezing weather is indicated as an alternative control measure. It is also evident, and in agreement with the experiences of the author, that shipment of firebrats for scientific purposes should take place only during the warm months of the year.

It may, too, be of practical importance to know that the use of temperatures of 50 to 60°C. (122 to 140°F.) for twenty-four hours or more, sometimes recommended for the control of insects of the household and stored products, would also be fatal to *Thermobia domestica*.

It should be noted that the firebrats used in the thermogradient studies above had been reared at 37°C. and that their preference of temperature may have been influenced by acclimatization. On the other hand, the firebrats tested at extreme temperatures were transferred quite abruptly from the favorable, to the unfavorable, conditions.

VI. CONCLUSIONS

It may be concluded that, for Thermobia domestica reared under favorable conditions at 37°C., and a relative humidity of about 70 per cent, the temperature relations are about as follows:

1. The fatal maximum temperatures for the firebrat, like those of most insects, are near 48 or 49°Cent. (118 to 120°Fahr.), the older individuals being the more resistant.

2. The maximum activative temperature, as here defined, was very close to the fatal maximum, and varied from about 46 to 47°C., depending on the individual.

3. The maximum completive temperature, as here defined, was between 42 and 45°C., probably about 43°C. (109°F.).

4. The thermotactic optimum was about 37.5°C. (99.5°F.) for the mature firebrats used in this study. Rearing experiments indicate that the general optimum is close to this point.

5. The minimum completive temperature was between 29.5°C. and 24.5°C. probably about 27.5°C. (81°F.) which incidentally is near the general optimum stated for many

insects.

6. The minimum activative temperature, as here defined, was near 15°C. (59°F.).

7. The fatal minimum temperature was indicated by inconclusive tests to be just below -7°C. (19°F.), the early nymphs usually proving more cold-resistant than the adults.

VII. SUMMARY

The firebrat, Thermobia domestica (Pack.), has been recognized as a heat-loving insect ever since it became known to science over 60 years ago. It is restricted to hearths, bakeries, and other places where temperatures above 30°C. prevail. In contrast to the temperature relations of the firebrat those of the larvae of the corn borer, Pyrausta nubilalis Hubn., are cited from unpublished studies. The borer thrives at ordinary summer temperatures near 27°C. The larvae were killed in laboratory experiments by one-hour exposure in air at temperature of 48°C. and survived prolonged exposure to temperatures in the winter of southern Ontario. Experiments upon the firebrat were aimed at determining its maximum, and minimum, fatal temperatures, the range of its preferred temperatures, and its thermotactic optimum. The latter is defined as the temperature the insect is most likely to choose by thermotactic responses when offered access to a range of suitable environments differing only in temperature. A thermogradient was constructed the principle part of which was a metal trough divided into transverse compartments separated by narrow openings. Each compartment was covered and so equipped that the insects might live in it indefinitely if suited with the temperature. When heat was applied at one end a rough, variable gradient of temperatures was obtained in the succeeding compartments. Firebrats in such a device

showed that they strongly avoided temperatures outside the range of 32 to 43°C. The mean point of the distribution, which is termed the thermotactic optimum, was 37.5°C. In other experiments it was found that firebrats would not breed at 24.5°C. and only very slowly at 29.5°C. Oviposition occurred at 42°C. but not at 45°C. Eggs at 37°C., near the preferred temperature, hatched in 13 or more days, and at 42°C. in 9 or more days. The life-cycle from egg to egg was at least four weeks shorter at 42°C. than at the preferred temperature. In a variety of preliminary tests the nymphs of the second instar and fully grown firebrats were removed from their favorable environment in the incubator and subjected to extreme temperatures. It was found that exposure to -7°C. for one hour, or to 2°C. for less than 24 hours, was sufficient to kill nearly all the animals so tested. Firebrats can live for many days at 45°C. and adults can live actively at 47°C. for many hours, although nymphs are slightly less resistant. An exposure to 49°C. for one hour is usually fatal to all firebrats. Thus the firebrat, so exceptional in its ability to live actively at 45°C. for days, has a thermal death point just under 50°C. like the European corn borer and many insects tested by others.

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PART THREE

THE GREGARINE PARASITES OF THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD)(THYSANURA)

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I. INTRODUCTION

In a previous publication (Adams and Travis 1935) two new species of septate gregarines parasitic in the firebrat, Thermobia domestica (Packard) (Thysanura), were briefly described under the names Lepismatophila thermobiae and Colepismatophila watsonae. In the present paper additional material is presented bearing upon the biology of these protozoa and their relations to their insect host. The aim of these studies has been to explore the general features of the problem and to establish some basic facts and methods helpful to further research.

It is believed that the firebrat and its gregarines are particularly well adapted to experimental use by entomologists setting out to study the obscure problem of gregarinosis in insects; and likewise by protozoologists concerned with certain problems of gregarine biology.

There remains a considerable variety of opinion among various authors as to the best classification of the Telosporidia. It is generally accepted, however, that the gregarines, in contrast to the coccidia and haemosporidia, form a natural group in which the mature trophozoites are extracellular, the zygotes are non-motile, and the sporozoites are enclosed in a sporocyst. The gregarines are divided by Legér into two groups: the schizogregarines, in which schizogony occurs; and the eugregarines, in which schizogony does not occur. The eugregarines are further divided into two groups which have been variously named by different authors. The names given by Lankester in 1885 and adopted by Bhatia (1924 and 1930) are also adopted here. According to this scheme the first of these (Acephalina, Monocystidea, of other authors) is Haplocyta Lankester and the second (Cephalina, Polycystidea of other authors) is Septata Lankester. The term "septate gregarine" is here used to designate a eugregarine in which the endoplasm of the trophozoite becomes divided into two portions (protomerite and deutomerite) by an ectoplasmic septum.

The terms for structures and processes of the life cycle conform with those in general use for Telosporidia, for the most part originated by Schaudinn. For convenience, the word "spore" is employed for the sporocyst with its contents, and the word "cyst" for the gametocyst with its contents. For the brief stage in which the developing parasite is neither a sporozoite nor yet a two-chambered trophozoite, the term "pre-septate" is proposed.

II. THE LITERATURE UPON SEPTATE GREGARINES

A survey of the literature upon sporozoa to the end of 1933 shows that there are at least two hundred publications extant which contain original data upon the septate gregarines. A bibliography upon the entire gregarine group arranged according to years, dating from the time of Redi to 1903, was compiled by Lühe (1904). The principal treatise upon the septate gregarines is the "Synopsis of the polycystid gregarines of the world" by Watson (1916) and Watson-Kamm (1922). Most of the literature up to 1914 is listed in these monographs, although some non-taxonomic works are omitted. The purpose here is to present a survey of the more important contributions of the past twenty years together with some of earlier date not listed by Watson-Kamm. Many papers which seem quite unrelated to the present study of firebrat gregarines are left unmentioned.

A. Taxonomy

As stated above the monographic work by Minnie Watson Kamm (appearing in two parts: Watson, 1916, and Kamm, 1922) constitutes the principal comprehensive work upon the species and classification of septate gregarines. This author has collected and re-written within the compass of a handbook the descriptions of the known species and has re-drawn hundreds of figures from the various authors. There are chapters upon the morphology and biology of gregarines, host-parasite lists, bibliographies, and a glossary. The first volume covers the species from Myriopoda, Orthoptera, and Coleoptera; and the second, the species from hosts in the rest of the animal kingdom. Of especial value is the classification of the genera of cephaline gregarines, with type species designated. (About fifty-seven of these genera clearly belong to the Septata.)

Of particular interest is Kamm's treatment of groups which are apparently transitional between the non-septate and the septate gregarines. Such are the Lecudinidae and Polyrhabdinidae, families created by Kamm for certain primitive epimerite-bearing forms parasitic in polychaetes. The first of these families consists of non-septate forms (which have since been included in the Haplocyta by Bhatia, 1930), and the second consists of more or less primitive septate forms (which must be included in the Septata). Since the most highly complex gregarines are found parasitic in the higher arthropods, which are commonly regarded as evolving from annelid-like ancestors, Kamm sees here an evolutionary parallelism between parasite and host. This view is further strengthened by the closely restricted distributions of certain gregarine genera in certain arthropod orders.

Despite its many typographical errors and small inaccuracies the work of Watson-Kamm is an invaluable manual for those engaged in studies upon gregarines.

Since the appearance of Kamm's monograph over a dozen genera and many new species have been established by various authors. The largest contributions have been made by Pinto (1922) and Tsvetkov (1929). Henry (1933) has created a new family, Kofoidinidae, to receive a new gregarine from a termite. The systematic positions of several new species have been left undecided (Vincent, 1924, *et al*). No general revision of the Septata has been attempted, although some recent contributions have been incorporated into text-book treatments (Doflein and Reichenow, 1929, and Calkins, 1935). It is the opinion of the author that a great deal of co-ordinated study should be put upon new and known gregarine material before another revision of the group be attempted. This attitude is based upon the observation that for very many species the cysts and spores, so important in taxonomic diagnosis, are unknown.

B. Cytology and Physiology

The mealworm, *Tenebrio molitor* L. (Coleoptera), an insect of world-wide distribution in stored cereals, has proved a rich and convenient source of gregarines for use by those investigating gregarine biology. The following parasites have been obtained from its digestive tract.

Gregarina polymorpha (Ham.) Stein

Gregarina cuneata Stein

Gregarina steini Berndt

Steinia ovalis (Stein) Léger and Duboscq

Ophryocystis mesnili Léger (A schizogregarine)

Following the contributions of earlier authors have come intensive studies of the morphology and physiology of mealworm gregarines by Kuschakewitsch (1907), Schiffman (1919), Mühl (1921) and Milojević (1924). The last mentioned dealt particularly with the origin of the gamete nuclei of *G. cuneata*. Joyet-Lavergne, in a series of papers appearing between 1924 and 1931, has dealt chiefly with the cytoplasmic features: mitochondria, Golgi apparatus, lipoid bodies, etc. from various cephaline gregarines of Scolopendra and Tenebrio. This author has investigated the evidences of sexual differences in the sporonts as revealed by vital staining (1926); he has shown (1931) a correlation between the oxidation-reduction potentials and the sexes of the sporonts. Rey (1931) has continued this work, making quantitative measurements of these potentials by micro-injections of Clark's dyes into the sporonts.

Sokolow (1912) carried on an extensive series of tests of the effects of various concentrations of various electrolytes upon motion in gregarines. A large number of species of septate, and other, gregarines were tested, including most of those mentioned above. The effects of unfavorable temperature and the temperature tolerances of these parasites were also tested.

A few authors have stated their methods of cytological technique in detail, notably Mühl (1921) and Ray (1933). These authors and Sokolow (above) have given much attention to the controversial problem of gregarine movement. Ray, after studying the movements of Stenophora khagendrae in saline containing carmine particles, agrees with Watson (1916) and earlier authors that the motion is a result of waves of fibrillar activity, the mucous exudate serving in traction.

C. Gregarinosis

The problem of the effect of gregarines upon their hosts has been reviewed and discussed by Watson (1916: 16-20). The same author (Kamm 1917, 1918, 1920) has published the results of three studies of the relation of gregarines to host tissue. The forms Stenophora lactaria Watson and Cephaloidophora delphinia Watson, in which development is intracellular, were found to have distinctly injurious effects upon the host tissues, each trophozoite destroying one to many cells. In cross-sections of epithelium parasitized by the latter species she noted "small barren areas" apparently produced by the destruction of groups of cells. In contrast to this was the effect of Gregarina rigida (Hall) Ellis upon the intestinal epithelium of the grasshopper, Melanoplus differentialis Uhler. Here the parasite does not penetrate the host cell but develops an epimerite which is thrust between cells and serves as an anchorage. The cells adjoining the parasite are pushed aside by its growth but they do not seem to suffer other than mechanical interference. This agrees with the conclusion of Seidlecki (1901) who regarded the effect of another extracellular gregarine, Nina (Pterocephalus) gracilis Grebnecki, thus: "Il ne provoque dans l'épithélium que des changements purement mécaniques". Joyet-Lavergne (1925) working with the same parasite found that the cells of the host (Scolopendra cingulata Latr.) which were close to the cephalonts were abnormally rich in lipoid bodies, some of which attained diameters of two microns, similar to those found inside the cephalont. He saw this as a response of the cells to stimulation by the parasite. Since he found a similar condition in cells of this host parasitized by Adelina dimidiata (Schneider), he suggested that lipoids may have an important role in host-parasite relationships generally.

No case has been found in the literature where the effect of gregarinosis upon the general well-being and longevity of the host has been measured. Sumner (1933) after rearing larvae of the mealworm (Tenebrio molitor) was of the opinion that those grown from the egg, in Petri dishes, by themselves, grew less rapidly than those grown among the gregarine-bearing adults. This note would be more convincing if backed by experiments to prove that the adults do not, in some other way, effectually alter the environment of the larvae by their presence.

It is probable that carefully controlled tests of the effect of gregarines upon the health of their hosts have not yet been performed. Various authors, after observing or rearing insects in the course of gregarine studies, have stated that they saw no outward impairment of the host. Such was the conclusion of Blunck (1923) upon the gregarines of dytiscid larvae, and of Henry (1933) upon certain gregarines of termites. Vincent (1922) made the interesting discovery that, although many of the cells of the intestinal diverticula of the beetle, Anobium paniceam L., contain symbiotic yeasts, those containing the epimerites of Pyxinia anobii Vincent did not. She stated that heavy gregarine infection over a period of five years had not seemed to impair the health of the insect culture.

Of particular interest is the work of Nowlin (1922) who has correlated the life-cycle of the gregarine, Schneideria metamorphosa Nowlin, with the metamorphosis of its host, Sciara coprophila. The parasite undergoes a polycystid development within the epithelial cell of the host larva; a non-septate sporont emerges from the cell and development proceeds no further until the pupation of the host, during which the sporonts unite in cyst-formation. The formation of spores is completed in the adult fly.

Bush (1932, 1933) has applied statistical theory to the measurement of gregarines. He contends that in the diagnosis of gregarine species, the addition of data upon the relative (heterogonic) growth of the parts of the animal would greatly increase the value of absolute measurements.

D. The Cyst and the Life-cycle

The details of the exogenous development of gregarines are highly various. In Leidyana species Watson (1916, p.39) has observed gametocysts, from the earliest stages of formation to the point where spore-ducts are already formed, all contained within the intestine of the host. Smith (1929) states that the cysts of Tettigonospora (Coccospora) stenopalmati Smith show no close correlation between age, with reference to passage from the host, and the stage of nuclear development. It is evident, however, that, in the majority of gregarines reported upon, the cysts are extruded while the contained gametocytes are still separated by a hyaline layer. In such cases the period which elapses between the passage of the cyst and the dehiscence of the spores varies greatly in different gregarines, and under different conditions. Chakravarty (1934), in India, found that this period for Stenophora ellipsoidi Chak. was two to three days. Daviault (1929) found the cysts of Leidyana ephestriae Dav. maturing in three to four days. As will be shown below, the author has found temperature a very important factor in these processes.

The moisture relations of gregarine cysts have received little attention. Ellis (1914) presents a very interesting account of the ability of the delicate cysts of Stylocephalus giganteus Ellis to survive desiccation. When kept in water these cysts gradually turn from white to gray and finally to black and extrude their spores in eight to ten days. Ellis allowed these fresh, white cysts to dry and shrivel for nearly five months in the dry, heated air of his laboratory in Colorado. When replaced in water these cysts promptly swelled and proceeded rapidly with spore-development.

A few writers have reported upon lengths of the life-cycle. Smith (1929) found that feeding carnivorous crickets, of the genus Stenopalmatus, with apple produced a purgative effect, making for rapid extrusion of gregarine cysts. He was able to secure passage of the parasite through the host in as little as twelve days although the average time was twenty-three and one-half days. Allowing ten days for the exogenous processes the period of the life-cycle of Tettigonospora stenopalmati was then about thirty-three days.

The spore of the gregarine is usually regarded as the most resistant form which gregarine protoplasm assumes. Smith (1930) found that the spores of the above-mentioned gregarine of the cricket lost their infective power between five and six weeks after escape from the cyst. The number of spores per cyst was placed at five thousand.

Among the miscellaneous papers is the first record of a gregarine from Hymenoptera: Bhatia and Setna (1924) described Leidyana xylocopae, from a carpenter bee. Hesse (1926) has reviewed the gregarines showing secondary segmentation. Keilin (1927) has shown the biotic position of certain gregarines in the fauna of a horsechestnut tree. A most unusual gregarine, of uncertain systematic position, has been described, by Ray and Chakravarty (1933), from a diplopod. In this form the sporonts entering into cyst-formation temporarily interlock with each other by means of forking tails, or pseudopodia, and there is a large, single, spore-duct (Monoductus).

E. Gregarines from Thysanura

The gregarines (all septate) which have been described or reported from members of the families Machilidae and Lepismatidae are listed here, together with their hosts: *Hyalospora affinis* A. Schneider, from *Machilis cylindrica* E. Geoff.; *Hyalospora roscoviana* A. Schneider, from *Machilis maritima* (Leach); *Gregarina lagenoides* (Léger) Labbé, from *Lepisma saccharina* Linn.; Gregarine "A", Cornwall, from Lepisma species; Gregarine "B", Cornwall, from Lepisma species; *Lepismatophila thermobiae* Adams and Travis, from *Thermobia domestica* (Pack.); *Colepismatophila watsonae* Adams and Travis, from *Thermobia domestica* (Pack.).

The first three members of the above list are discussed by Watson (1916, pp.122, 166) and Kamm (1922, p.54); the last two are subjects of this paper. The gregarines reported by Cornwall (1915) do not appear in the protozoological literature; they deserve special mention here. Cornwall worked with an insect in India, which he took to be *Lepisma saccharina* but whose identity he was not able to confirm. In the course of studies upon its biology, he found two species of gregarines occupying the anterior part of the ventriculus in most of his specimens. The two were widely different in character. He termed them "A" and "B".

The facts regarding these parasites, based on Cornwall's data and drawings, may be briefly reviewed. Species "A": Trophozoites, large, easily visible to naked eye; deutomerite elongate, conoid, wider than the thimble-shaped protomerite;

the epimerite large and globular, containing a delicate transverse septum. Sporonts solitary. Cysts globular, becoming shrivelled, darkening as spores mature. Spore black, with thick, finely pitted sporocyst; elongate oval, with greater curve on one side than the other; dehiscing along the line of lesser curvature; escaping in convoluted chains from the ruptured cyst. The septa, between deutomerite and protomerite, and between protomerite and epimerite, are described as quite marked. The trophozoite is figured as occupying a deep cavity in the epithelium; the epimerite is thrust nearly to the basement membrane and the protomerite crowds the surrounding cells. The spore is described as having in the center a globule, and in each end four shapeless sporozoites.

The species "B": Much smaller than "A". Trophozoites roundly heart-shaped, with a long, acicular epimerite arising from the emargination and penetrating the epithelium to the basement membrane. Sporonts "assume peculiar shapes in the course of intimate association with each other without actual fusion". Cysts tiny, just visible with 10x lens; remaining white and dehiscing white spores, in chains, from spore-ducts which are about as long as the diameter of the cyst. Spores about four microns in length and two microns in width, symmetrically curved, and joined end to end. Early trophozoite entirely intracellular, causing hypertrophy of the cell and distortion of the nucleus.

Species "B" is very evidently quite unrelated to Lepismatophila and Colepismatophila; but it is equally evident that species "A" has most of its characters much like those of these firebrat gregarines. Both "A" and "B" are quite different from Hyalospora, in which the sporonts are narrowly cylindroid, and from Gregarina, in which the sporonts exhibit typical syzygy.

An investigation of these forms by a worker familiar with gregarines would undoubtedly bring forth two new species, if not new genera, and much valuable information.

Gregarines from the firebrat have hitherto been observed by at least one investigator. Spencer (1935), in a private communication to the author, stated that in his firebrat cultures gregarines had at first been abundant but that, with the passage of a few years, these parasites had become very scarce and had seemed to occur only in the ill-nourished specimens. Spencer did not identify or describe these forms but confined his studies to the host.

III. OBSERVATIONS AND EXPERIMENTS

A. History of the Study

The cultivation of firebrats, *Thermobia domestica* (Pack.), under conditions of controlled temperature and moisture, has been carried on at Iowa State College for a number of years. The biology and methods of rearing have been outlined by Adams (1933). The cultures were found to contain two species of gregarines which were described in a brief paper by Adams and Travis (1935) and designated as types of two new genera for the family Gregarinidae. The firebrat proved to be a very satisfactory host animal for laboratory use and experiments upon the biology of its gregarines have followed.

B. Organization of the Firebrat as a Host

The external morphology of lepismatids has been described by Escherich (1905). The mouth parts are a generalized, mandibulate type. The maxillary and labial palpi are well developed. The schlerotized teeth of the mandibles are effective in scraping food-bearing (and spore-bearing) surfaces, and for carrying detachable morsels of food. A pair of salivary glands in the thorax are in communication with the buccal cavity and doubtlessly have an important rôle in the taking of dry food.

The alimentary canal is only slightly longer than the body (Fig.10). The thin-walled crop extending throughout most of the thoracic region makes up about half the total length. This organ is usually distended with a watery fluid containing food particles in suspension. The crop, when dissected out in saline, persists in a rhythmic peristalsis. The muscular proventriculus or gizzard contains six schlerotized teeth. There is a constriction at the region of union of fore-gut and mid-gut.

The ventriculus makes up a greater part of the remaining length of the food canal. The short, rounded, caeca usually number about fourteen. They communicate freely with the main lumen and may be directed forward, laterally, or backward, in the body of the animal. The cells of the ventricular epithelium are deeply columnar in the caecal region but become shallower and more evenly placed in the middle and posterior portions of the ventriculus. Thus, posterior to the caeca, the cross-sectional outline of the epithelium becomes smoothly circular. A peritrophic membrane separates the food bolus from the epithelial cells, providing a space for parasites. The ventriculus becomes more muscular in the posterior half and here peristaltic bands are noted in living specimens.

The Malpighian tubules are few and very long (10-20 mm.).

The hind-gut is shorter than the mid-gut and the rectal epithelium is thrown up in six weak folds.

C. Methods and Technique

Methods of rearing the host are discussed elsewhere (Adams 1933). In order to maintain colonies of firebrats free from gregarines or with one or the other species of gregarine in pure culture it is necessary to prevent contamination by unwanted spores. Instruments and containers are disinfected by exposure for an hour or more at 80°C. or by wiping the suspected surfaces with strong ethyl alcohol. The largest culture dishes used are about eight centimeters in depth and of various diameters. The lids are placed on, but they are slightly raised by wire supports to permit circulation of air.

Firebrat colonies are inoculated by introducing spores mixed with their food. Infective mixtures are prepared by shaking together cereal food and feces from infected animals. Where a firebrat is to be infected, and observed, singly, the insect is starved for a few days and then presented with a fragment of a rolled oat bearing a known number of spores. For such studies small, deep Petri dishes are satisfactory.

The collection of gametocysts is facilitated by housing infected animals in a chimney-like glass cylinder containing roosting sheets (sheets of paper plaited and stood on edge so that the plaits are vertical). This device stands in a shallow dish into which the feces drop. The transfer of the cysts is facilitated if the tip of the needle bears a moist film of insect haemolymph.

In studying the trophozoites in vitro it is important to completely remove them from the tissues of the host (which quickly putrify) and immerse them in a fluid of suitable tonicity, which, for these species, is a one-per-cent solution of sodium chloride. It is extremely important that the salt concentration should be held at this point. Slide preparations must be sealed if the parasites are to be observed in motion for more than half an hour.

For the study of the endogenous cycle and the relations to host tissues, the guts of insects of known infection history were removed entire into saline and fixed in Zenker's fluid for 4 to 24 hours, cleared in xylene, embedded in paraffin (melting point 54°), and sectioned at a thickness of eight microns. The sections were stained in Ehrlich's haematoxylin, counterstained in eosin, and mounted in balsam. This method, while satisfactory for the insect tissue, is not unconditionally recommended for gregarines.

The gametocysts have not yet been satisfactorily sectioned. Smears of the cysts were prepared by crushing them upon albuminized cover-glasses, fixing in warm Schaudinn's fluid, staining in Heidenhain's iron-haematoxylin, and differentiating in iron-alum mordant.

D. Lepismatophila thermobiae Adams and Travis

The description of the trophozoite, and other stages of this species, by Adams and Travis (1935) was brief and strictly diagnostic in character. Fuller descriptive data are presented here.

1. The trophozoites

The smaller cephalonts (Figs.4 and 5) have the deutomerite broadly conoid and the protomerite hemispheroidal. The septum, near which the greatest width usually occurs, is nearly plane or slightly concave caudad. The epimerite is a globose, sessile knob. The nucleus is spherical.

The measurements of trophozoites in this paper were obtained from camera lucida drawings of living specimens in saline solution just after removal from the tissues. The particular measurements given for this species are chosen as representative from 125 specimens so studied. The widths are taken at the widest part, at right angles to the long axis of the animal. The measurements for some smaller cephalonts are given first (Table I).

Table I

Measurements in Microns of Five Smaller Cephalonts

Total length	116	106	98	67	52
L. Protomerite	24	18	24	15	9
L. Deutomerite	92	88	74	52	43
W. Protomerite	49	64	43	30	18
W. Deutomerite	55	64	49	46	24
D. Epimerite	18	21	12	12	—

Legend: L = Length, W = Width, D = Diameter.

The larger cephalonts and smaller sporonts (Figs.3 and 6) have the deutomerite relatively longer and more tapering. The epimerite, which is easily detached, is a smooth, globose, sessile, knob; either hyaline or with a sparse granular content. The protomerite has a hyaline ectoplasm, wider than that of the deutomerite; the small, rather coarsely granular endoplasmal mass has a very pale straw-color by transmitted light. The endoplasm of the deutomerite is more finely and closely granular, presenting a denser, more whitish appearance. In some stages of movement the posterior end of the deutomerite becomes constricted and prolonged into a more or less abrupt tail. The greatest breadth of the deutomerite is usually across the anterior fifth of its length.

The larger sporonts, or adults (Figs.1 and 2) are often found in small numbers in the post-caecal region of the mid-gut where they can be seen in situ, as densely white bodies, visible through the nearly transparent, ventricular wall. The mature sporonts are relatively broader and more rounded than the cephalonts and the deutomerite is relatively fuller. The nucleus is seen as a spherical light area in the dense endoplasm; it averages about 31 microns in diameter. In living specimens the septum is often obscured by the endoplasmal masses,although the parted condition of endoplasm, peculiar to septate gregarines, may still be indicated by lateral indentations. The protomerite in this stage is more cap-shaped, i.e., the septum is convex cephalad. The measurements of some larger sporonts are given in Table II.

Table II

Measurements in Microns of Five Larger Sporonts of
Lepismatophila thermobiae

Total length	391	317	268	372	360
L. Protomerite	43	55	31	55	49
L. Deutomerite	348	262	237	217	211
W. Protomerite	122	153	92	110	152
W. Deutomerite	134	146	85	110	152
Ratio of L. of Protom. to total L.	1:9.1	1:5.8	1:8.6	1:6.7	1:7.3

Legend: L = Length, W = Width.

The trophozoites living contemporaneously in one ventriculus sometimes range very widely in size. Usually, however, a gap is found in the size-distribution, indicating separate infections. The measurements of five living specimens, removed to saline from one host animal and selected to show the range in size, are given in Table III.

Table III

Measurements in Microns of Five Trophozoites of
Lepismatophila thermobiae

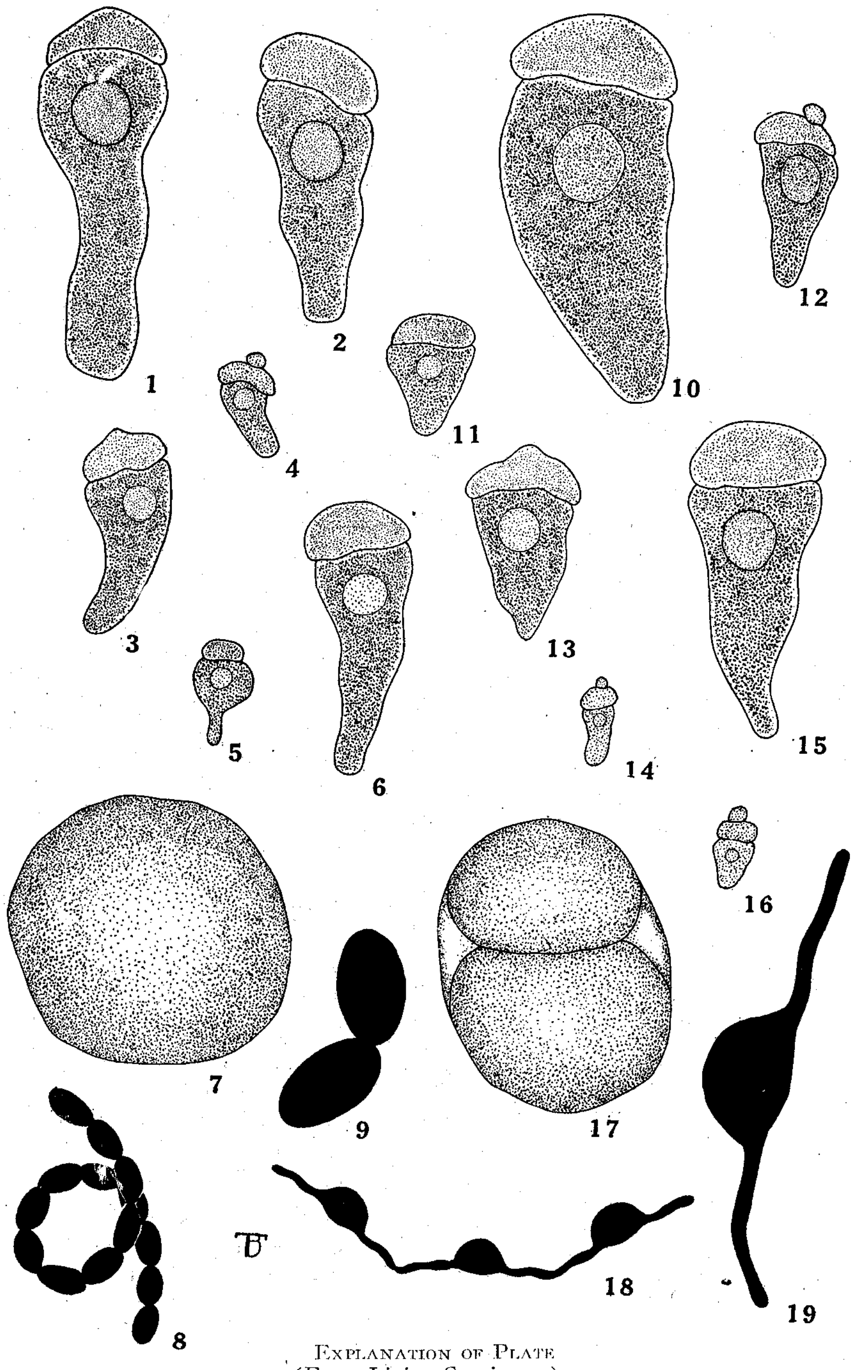
Removed from One Firebrat

Total length	335	287	250	183	91
L. Protomerite	49	48	31	18	21
L. Deutomerite	286	239	219	165	70
W. Protomerite	153	153	104	157	37
W. Deutomerite	159	183	110	174	55
D. Epimerite	24	—	—	18	12

Legend: L = Length, W = Width, D = Diameter.

The trophozoites of this gregarine are indistinguishable from those of Colepismatophila watsonae. The species is distinguished by the absence of appendages upon the sporocyst and the position of the cephalonts in the ventricular caeca. Where both species have been present in the same culture of host animals, L. thermobiae has been the more abundant. The largest number of trophozoites recorded in an individual firebrat is about two hundred and forty. More commonly the number in an infected animal is less than fifty. In heavily infected cage lots nearly half the individuals have been found harboring trophozoites of this species at one time. The larger trophozoites are readily visible through the straw-colored, semi-transparent caecal wall. By reflected light they appear as whitish masses. When teased out into distilled water, or hypotonic saline (such as 0.8 per cent), the parasites become swollen and the hyaline layer, between the endoplasmal masses of the deutomerite and the protomerite, widens until the organism bursts. In tonic solution (1 per cent) these gregarines have retained, at room temperature, the power of locomotion for six to eight hours, and they have remained in sealed preparations for twenty-four hours without visible cytolysis. The locomotion of sporonts consists of a cephalad gliding movement similar to that described for other gregarines. One sporont in saline moved its own length in seven minutes. The bending of the body upon its own axis results in changes of direction. The presence of mucus upon the trophozoite's body is evidenced by the readiness with which, in dissections in saline, the caudal end of the deutomerite becomes bound to bits of débris.

The sporonts of this species are solitary. The phenomenon of syzygy, common in species of the genus <u>Gregarina</u>, has not been observed in <u>Lepismatophila</u>. It should be remarked that the body-forms in the sporonts of many Gregarina species are truncated, whereas those in the sporonts of <u>Lepismatophila</u> remain conoid and probably somewhat unfavorable, therewith, to end-to-end associations. Only the largest sporonts are found posterior to the ventricular caeca. Occasionally a host speci-



EXPLANATION OF PLATE
(From Living Specimens)

Lepismatophila thermobiae sp. nov.: Figs. 1–6, trophozoites X125; 7, cyst X125; 8, spore-chain X300; 9, spores X1200.

Colepismatophila watsonae sp. nov.: Figs. 10–16, trophozoites X125; 17, cyst in early stage of formation X125; 18, spore-chain X300; 19, spore X1200.

Figs.1-19. From Adams and Travis. Reprinted from the Journal of Parasitology, 21:57. 1935.

men is found to contain, in the hinder part of the ventriculus, a pair of more or less rounded sporonts lying in close proximity to each other, or in contact, plainly entering upon pseudoconjugation. It is evident that the mature sporonts migrate from the caeca toward the rear of the ventriculus and that, upon finding partners, they lose their conoid form and become rounded for cyst-formation.

Experience in rearing has shown that the life-cycle from spore to spore at 37°C., and under other conditions favorable to the firebrat, is commonly completed in three weeks. In one case, however, a shorter cycle was obtained. Twelve firebrats were fed spores and their feces examined frequently for gametocysts. One cyst appeared on the tenth day and two more on the thirteenth day. Allowing four days for spore development at this temperature the minimum observed life-cycle period from spore-feeding to spore-production is fourteen days. It seems probable that tests with hundreds of specimens, under varying conditions, would show even a shorter cycle, as short as those of Colepismatophila watsonae.

Infection with Lepismatophila thermobiae occurs in small, as well as fully grown, specimens. Nine nymphs between 3.5 and 5.0 millimeters in length, mostly still bearing only one pair of styli, were dissected, after being exposed to spore-bearing food since hatching. One had twelve small cephalonts, one had fifteen cephalonts ranging from 180 to 200μ in length, and one had two cephalonts in the caeca and one sporont 230μ in length in the posterior end of the ventriculus. In the others no gregarines were found. The culture dish contained several remnants of erupted cysts from earlier infections.

No statement can be made regarding the effect upon the host save to say that firebrats, as seen in the casual observations of experience in rearing, seem to thrive about equally well with and without these gregarines. Examination of serial sections of infected ventricular caeca showed the trophozoites to be more or less closely packed into the crypts and folds of the epithelium. In some cases the trophozoites seem to have made small pockets for themselves. There was, however, no observed case in which the protomerites extended as deep as the basement membrane, or completely displaced patches of epithelial cells, as do those of the next species.

2. The cysts.

Cysts, in a very early stage of development, are occasionally found in the posterior end of the ventriculus of the host. In some observed cases the contained gametocytes had not yet changed from their spheroidal form and the clear, colorless cyst wall (cyst proper) was very thin. A similar case has been observed in Colespismatophila (Fig.17). The contained pseudoconjugants become progressively flattened against each other until they are almost hemispherical leaving a hyaline layer between them which becomes almost perfectly disk-shaped, its thickness sometimes exceeding one-tenth of the length of the cyst. This hyaline layer is on the plane perpendicular to the longer axis of the cyst.

The cysts escape from the host in close association with the feces. As a rule, the cysts are found shallowly pressed into the fecal particles, from which they are readily removable. One pellet was observed to bear, along its sides, no less than eight cysts of this gregarine. Cysts passed simultaneously, that is, upon the same fecal pellet are not always at the same stage of development. In some cases the gametocytes are not fully flattened upon the hyaline layer between them; but in the majority of freshly dropped cysts the hyaline layer is fully discoidal, and the gametocytes hemispheroidal.

In the first few hours after the cyst is passed by the host the gametocytes remain sharply distinct. The cyst wall, although increased in thickness, is still colorless and nearly transparent. The pearly white color of the whole cyst is chiefly due to the whiteness of the contained gametocytes. In shape the fully formed cyst is usually ellipsoidal, the gametocytes occupying the ends. Ovoidal and spheroidal forms are also observed (Fig.7). The average of the measurements for twenty cysts were: for the longer axis, 297µ; and for the shorter, 214μ. The largest cyst in this series measured 378 x 262μ; the smallest 244 x 171μ.

Within the day (at 34.5°C.) after the passage of the cyst the hyaline layer between the gametocytes becomes narrowed and finally obliterated. The exact time of the intermingling of the gametes from each side has not been determined, although fixing and clearing agents have shown the gametocytes to be still intact, and entirely distinct from each other, some hours after they have closed in upon, and covered or absorbed, the hyaline layer between them. On the second day the cyst is solid white; and on the third it is grayish, owing to the pigmentation of the forming sporocysts. On the fourth day the cyst becomes very dark gray, and on the fifth, intensely black. The blackness of the ripe cyst is increased by a dull grayish-brown coloration of the cyst wall which appears in the ripening process. The shape of the cyst is often modified, late in the period of spore-development, by the appearance of a flattened, or even a concave, area upon one side. This change is probably due to shrinkage of the contents resulting from loss of moisture, for it can be increased by lowered humidity; it is most marked in cysts which fail to dehisce spontaneously. Dehiscence of the cysts begins on the fifth day.

The dehiscence of scores of cysts has been observed. Under the most favorable conditions the cysts, when ripe for dehiscence, are plump and very black. The cyst wall, spontaneously or at the touch of the needle, suddenly breaks open and in a few seconds becomes everted releasing the helical spore-chains (Fig.8) which rapidly uncoil and extend away from the ruptured shell, in various directions, until they form a black, fluffy, mass, covering an area several times wider than the diameter of the cyst. The chains continue to uncoil and extend for an hour or more,until the ball of spores is completely dissipated. The remnant of the cyst, with its ragged, recurved edges, flattens out beneath the extruded mass. The expanded, loose, mass of spore-chains can now be entirely separated from the remnant of the cyst. The latter is seen to consist of a thickened central portion (residual protoplasm?) surrounded by radiating sectors of the torn wall, which is tinted with a brownish-gray color and contains a net-work of bold, pigmented channels, or lines.

While the eversion of the normal cyst is too rapid to be observed in detail, some understanding of the process may be obtained from observation upon cysts in which dehiscence is retarded by low temperature or low humidity. Here, the rupture of the cyst wall begins with the appearance of a small hole, or pore, from which the disrupted edges extend outward as though the wall were punctured from the inside outward by a pointed instrument. Through this opening the black spore mass is readily visible. In some cases of feeble dehiscence more than one opening of this kind may appear in the wall without the immediate release of the spores.

The dehiscence of the cyst and the release of the spores seem to involve two agencies: (1) the tendency of the coils of spores to elate and (2) the tendency of the cyst wall to evert itself. It is evident that these tensions must arise in the joints of the spore chains and in the cyst wall itself, as the time for dehiscing approaches; only the cysts which are quite black exhibit these phenomena.

The number of spores from the cyst is estimated as varying from two to four thousand.

The temperature relations of the firebrat are quite unusual since the insect actively prefers a temperature near 37°C., can endure 46°C. for days, and can scarcely complete its life-cycle at ordinary room temperatures. It was a question, then, what the temperature relations of its gregarines might be. At a temperature of 34.5°C. and a relative humidity between 60 and 70 per cent, dehiscence of the cysts usually occurs in 110 to 120 hours after their escape from the host. At 43°C. dehiscence began on the third day. Firebrats held at 45°C. produced cysts during the exposure to this unfavorable temperature. These cysts did not seem to be impaired by the heat for, when moved to an incubator at 34.5°C., they darkened and dehisced in a few days. A few cysts produced at 46.5°C., at low humidity, darkened but did not dehisce. Firebrats, moved from air at 34.5°C. to air at 24.5°C. of about the same humidity, produced cysts for weeks, but at a greatly reduced rate. Most of these cysts became black in ten days but none dehisced in three weeks. Of sixteen cysts produced at 34.5°C. and set at 24.5°C. while fresh, a few feebly burst in twelve to sixteen days.

3. The spores.

The chains of spores (Figs.8 and 9) lie intact about the remnant of the dehisced gametocyst until disrupted by outside forces. When touched by the needle the chains readily break up into short, fragmentary chains which adhere closely to the food of the host, to the bodies of scaleless nymphs, and to the investigator's needle.

The length of time through which spores might retain their power to infect the host was another point of enquiry. Adult firebrats, from gregarine-free cultures, were placed in groups of five in small rearing dishes and fed rolled oats which had previously been shaken with feces containing spores of known ages. The feces had been collected at various times and had been stored in loosely corked bottles on the laboratory shelf. The spore-bearing samples were of two, three, six, eight, and seventeen months duration. Observation on these groups of animals were continued until cysts appeared in their feces or until the elapse of three months, after which the ventriculi of the animals were inspected for trophozoites. Two series were run at different times. As to the results: only the firebrats infected with the spores which were three months or less of age became infected.

Studies upon the microscopic morphology of the sporocyst and the manner of attachment in the chain have not been completed. A central, refractile body in the spore is sometimes visible through the pigmented sporocyst. This is probably a residual body. The form and position of the sporozoites in the spore has not been ascertained. The material obtained from crushed spores was quite formless.

4. The sporozoites.

Attempts were made to observe the trophozoites *in situ* within the spore. For this purpose the sporocysts were bleached with strong hydrogen peroxide and other agents. Attempts were made to induce the sporozoites to leave the sporocyst *in vitro* in saline, in the presence of bits of ventricular tissue from the firebrat. Crushing the sporocysts was also tried. All these methods failed to disclose the shape and position of the sporozoites. It may be, however, that a system of fixing, bleaching, staining, and destaining, will be developed by which these bodies can be demonstrated in the sporocyst.

Cross-sections of infected ventriculi have not been studied sufficiently to warrant statements about the form and habits of the sporozoite in the tissues of the host.

E. Colepismatophila watsonae Adams and Travis

The discussion of this species is facilitated by comparisons with the species discussed above. The resemblances are very great; the chief differences are in the form of the spore (generic character) and the site of attachment in the host.

1. The trophozoites.

The cephalonts of this species (Figs.12, 14, 16, and 20) are found in the lumen of the ventriculus, in the anterior one - half of the length, posterior to the caeca. In rare instances one or two have been found in the caeca. The epimerite is a smooth, symmetrical, sessile knob; it is (according to histological sections) cylindrical when inserted into the tissues, but it is globose when withdrawn in the living state. The protomerite is hemispheroidal to conoid. The deutomerite is usually broadly conoid. The dimensions of some smaller cephalonts are given in Table IV. In this study 125 living trophozoites were measured. The measurements presented here were selected as representative.

One per cent saline was found a satisfactory medium for use in sealed preparations of living trophozoites.

Table IV

Measurements in Microns of Five Smaller Cephalonts of
Colepismatophila watsonae

Total length	116	116	103	98	73
L. Protomerite	30	37	40	31	18
L. Deutomerite	86	79	62	67	55
W. Protomerite	46	55	66	55	37
W. Deutomerite	49	67	61	55	37
D. Epimerite	21	18	12	23	12

Legend: L = Length; W = Width, D = Diameter.

The larger trophozoites (Figs.10, 11, 12, 13, and 15) are, as a rule, relatively broader than those of L. thermobiae. Otherwise the description of the latter applies to the present species. The trophozoites of this species seem to abandon their epimerites and live as sporonts at smaller sizes than do those of L. thermobiae. It seems likely that growing trophozoites living in the main lumen of the ventriculus, even though protected by the peritrophic membrane, are more apt to be disturbed by peristaltic action and the passage of food than are trophozoites lying in the diverticula. For this reason the trophozoites of *C. watsonae* may reach the sporont condition, and the power to move freely in accommodating themselves to varying pressures, at an earlier point in their growth than those of *L. thermobiae*.

The larger sporonts closely resemble, in color and in shape, those of *L. thermobiae*. Certain slight, but fairly constant, differences are shown by measurements (Table V).

Table V

Measurements in Microns of Five Larger Sporonts of *Colepismatophila watsonae*

Total length	315	348	384	439	562
L. Protomerite	49	73	61	98	111
L. Deutomerite	256	275	323	341	451
W. Protomerite	158	158	134	134	146
W. Deutomerite	171	153	177	189	183
Ratio of L. of Protom. to Total L.	1:6.3	1:4.7	1:6.3	1:4.5	1:5.1

Legend: L = Length, W = Width,

It will be noted that these sporonts are larger, and that the length of the protomerite averages a smaller fraction of the total length, than in the case of *L. thermobiae*.

The range in size in trophozoites found in one host at one time is represented by the measurements of five gregarines from one firebrat, given in Table VI.

Table VI

Measurements in Microns of Five Trophozoites of <u>Colepismatophila watsonae</u>

Removed from One Firebrat

Total length	354	268	128	116	92
L. Protomerite	79	67	37	37	31
L. Deutomerite	275	201	91	79	61
W. Protomerite	201	171	79	55	55
W. Deutomerite	226	183	79	67	58
D. Epimerite	—	—	18	18	18

Legend: L = Length, W = Width, D = Diameter.

In some cases the gregarines in a ventriculus are spread about throughout the space customarily occupied; in others they are closely crowded on a small, localized area of the epithelium, occupying less than one-half of the cross-sectional circumference. In a few instances these clumps of gregarines have produced a marked bulging of the infected portion. The early cephalonts, which are highly variable in form have only their short epimerites inserted into the epithelium; but the older sporonts were found, in cross-sections, to occupy deep pockets in the epithelium. In two cases the sporonts were so large that less than a dozen of them was sufficient to fill the lumen; and the pockets into which the parasites were inserted were so deep and broad that some of the protomerites touched the basement membrane and there was little space left between them for the epithelial cells.

In studies upon the portion of the life-cycle passed in the host, full-grown, uninfected, firebrats, in groups of thirty to forty, were starved for two days and then given access, for twenty-four hours, to food heavily contaminated with spores. The insects were kept at about 36°C. and at the humidity obtained over saturated sodium chloride solution containing an excess of the salt—about 70 per cent R.H. On each succeeding day, or more frequently, five individuals were removed and dissected. The alimentary tracts, whether showing gregarines or not, were fixed and sectioned. In one experiment trophozoites, large enough to be readily visible while being dissected out under thirty magnifications, were found in a specimen ninety-six hours after introduction of the spores. In another lot of insects treated in the same way the first trophozoites of such a size were found only sixty-six hours after introduction of the

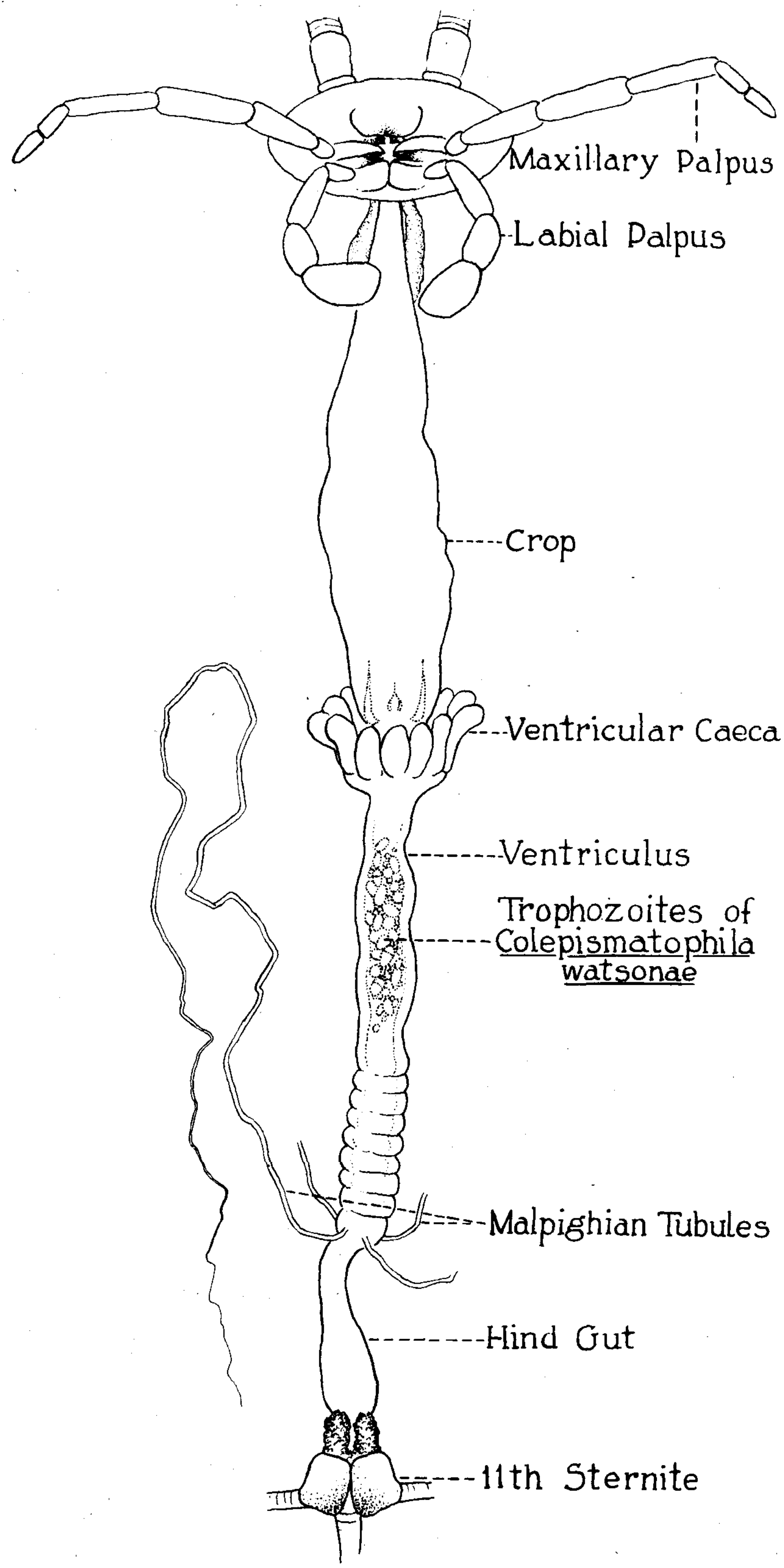


Fig.20. Alimentary tract of <u>Thermobia domestica</u>, with head and anal segment attached, showing position of gregarine parasites.

spores; and one of the remaining firebrats of this group produced a cyst early on the sixth day. The cyst was black by the end of the seventh day. Allowing one more day for ripening and dehiscence of the cyst this life-cycle in firebrats at 36°C., was a matter of no more than eight days duration. In another experiment twenty firebrats reared from the egg, without gregarines, were given access to spore-laden food and maintained at 33°C. In this case, again, cysts were found on the sixth day, or, after about one hundred and forty hours. On the seventh day fifteen cysts were collected; on the eighth day twenty-five cysts were dropped by the infected animals and, on the ninth day some of the earlier cysts were found dehisced.

Firebrats, in cultures having both species of gregarine, produce cysts of both species at the one time. Sixteen females were removed from such a mixed culture and kept singly in order that the spores from the cysts they produced might be identified as to source. In six weeks all these females produced spores; thirteen produced spores of both gregarine species.

It should be noted that this slow method of identifying the species concerned in an infection is entirely necessary, since single trophozoites of the two cannot be reliably distinguished merely by their position in the gut. While the cephalonts of L. thermobiae are nearly always confined to the caeca, the customary zone of attachment of C. watsonae begins immediately behind the caeca; and, in heavy, mixed infections, the ranges of the cephalonts, as well as of the sporonts, of the two species, are likely to become slightly overlapped.

2. The cysts.

The freshly dropped cysts are indistinguishable from those of L. thermobiae. There is, however, a marked difference in average sizes. The average of the long diameters of twenty cysts was 360µ; and the average of the short diameters was 232µ. The range in this lot was very wide: the largest measured 464µ in length and 336µ in breadth; the shortest, 226µ in length and 159µ in breadth.

The number of spores produced in a cyst varies with the size of the cyst. The spores from a cyst of C. watsonae are fairly easy to spread out and count under a squared, eye-piece micrometer. After making such a count upon portions of the spores from a few cysts the total number of spores in an average cyst was estimated to be near sixteen hundred.

The effects of environment upon the rate of developments within the cyst are closely like those stated for L. thermobiae. The cysts of this species are also passed at various early stages of development and reach the point of dehiscence in varying lengths of time. Some cysts, at 33°C., have been observed to reach the black stage in sixty hours after passage from the host. At 38°C. spores are released in about four days. Smears of the cysts made late on the first day show a variety of bodies whose identity have not yet been worked out. Smears of cysts late on the second day show the forming sporocysts pale and unconnected. The details of the exogenous cycle are a study in themselves.

3. The spores.

The sporocyst (Figs. 18 and 19) upon which the species have been separated generically, is black by reflected, and brown by transmitted, light. It consists of a hemispheroidal middle portion, a little larger than the whole sporocyst of *L. thermobiae*, which bears, extending in opposite directions from opposite sides of the periphery, two strong, twice-curved, filamentous, processes, by the tips of which the sporocyst has connections with the tips of the nearer processes of the adjoining sporocysts in the chain. The sporocysts have the outline of a broad-brimmed hat seen in profile. The dimensions of the middle portion, based on ten specimens, average 16.5μ for the long axis and 9.7μ for the transverse axis. Although there is very little variation in the dimensions of these sporocysts, there is much variation in the details of the curvatures of their processes. The processes averaged 21.1μ in length and 1.5μ in thickness. The spore chains of this species are not closely convoluted; their form may be described as wavy.

When fresh, they may be taken up in considerable lengths with the needle; but, when dry, they disintegrate readily to the individual spores. Spores of this species retain their power of infection for at least three months.

4. The sporozoites.

The sporozoites of this species, like those of the last, have not been obtained outside the host. Sections of the crops of animals which have been infected have shown both intact, and broken, sporocysts. Sections of the proventriculus have shown chiefly sporocyst fragments. Sections from the site of infection have shown minute, elongately ovoidal, bodies, each with a large, dark-staining nucleus and very little cytoplasm. These are situated, apparently, between the epithelial cells. They are regarded as being either the sporozoites or the earliest pre-septate trophozoites, although there is a considerable gap in sizes between them and the smallest indubitable trophozoites seen in the same sections. Various pre-septate trophozoites, from ovoidal to lanceolate in shape, have been observed in sections of the epithelium; the pointed end being thrust into the tissue and the rounded end projecting into the lumen. A fuller discussion of the sporozoites and early trophozoites forms a separate study.

IV. DISCUSSION OF RESULTS. SUGGESTIONS FOR FURTHER STUDY.

This paper is intended to lay the basis for more conclusive studies upon its various phases. The uppermost purpose is to present the species and their host as suitable material for experimental use - their merits being (1) the ease with which the insect is adapted to laboratory rearing, and (2) the speed with which the gregarine processes are completed at the biologically high temperatures at which the host must be reared.

In view of the close similarities of the two species it becomes doubtful to the writer whether it was wise to erect two genera merely upon the morphology of the spores. It is hoped that if both should be placed in the same genus in the future the name Lepismatophila will be preferred.

The problem of gregarinosis remains unsolved. It is apparent that Colepismatophila watsonae can partially obstruct the ventriculus and that it causes histolytic damage. But, since outward symptoms of disease were not observed, it is evident that the worker who would investigate this matter from the standpoint of firebrat survival would have to use very delicate symptomatic criteria, such as the effect of severe infection on the rate of the insect's oviposition or its food intake. Such experiments would demand extremely painstaking methods and thorough acquaintance with the animals concerned.

Another attractive problem arising in this study is that of explaining the great variation in the numbers of gregarines found in hosts in the same cage and the fact that a great many of the insects from the same cage contain no gregarines whatever when examined, while others have both old and young trophozoites. Is there an immunity? What factors limit the frequency and intensity of infection?

There is an opportunity here to test the effect of foods and of poisons upon host-parasite relations. The firebrat is quite omnivorous and might be fed a variety of deficient, or toxic, materials.

In addition to these, there are the usual protozoological problems here merely made evident: the cytology of the exogenous cycle; the nature and movements of the sporozoite; the cytology and physiology of the trophozoite; the relation of the trophozoite to the parasitized cell-groups; the habits of the sporonts in cyst-formation; the relations of the invading sporozoite, and the escaping cyst, to the peritrophic membrane; and many others.

V. CONCLUSIONS

Since most of this study is exploratory in character only a few general conclusions will be stated.

1. The firebrat, Thermobia domestica (Pack.) and its gregarines, Lepismatophila thermobiae and Colepismatophila watsonae, described by Adams and Travis, are, owing to the ease with which they may be reared continuously at biologically high temperatures, well suited to experimental use, particularly in research upon host-parasite relationships.

2. Further study of the two gregarines has not strengthened the basis for making them of separate genera.

3. The sporonts of Colepismatophila watsonae sometimes produce marked, local, histolytic damage to the digestive epithelium of its host, Thermobia domestica.

VI. SUMMARY

The gregarines of Thermobia domestica (Pack.), named below are numerous in cultures of the insect; and, like the insect, they are convenient for continuous laboratory rearing. Firebrats in cultures containing one or both species of gregarines seem to thrive as well as others reared gregarine-free from the egg.

Lepismatophila thermobiae Adams and Travis.

A fuller description is given. As many as two hundred and fifty trophozoites have been taken from the caeca of one firebrat. The mature sporonts move toward the posterior end of the ventriculus to encyst in pairs. When the cysts are deposited by the host the gametocytes are usually hemispheroidal with a discoidal, hyaline, layer between them. The latter disappears in the first day of the exogenous cycle. At 34.5°C. the cyst acquires a grayish tint on the third day, owing to the developing sporocysts. On the fifth day the cyst is intensely black and likely to burst, dehiscing a ball of convoluted chains of spores. At 43°C. dehiscence comes in three days. Spores retain their infective power for at least three months.

Colepismatophila watsonae Adams and Travis.

The trophozoites are crowded between the peritrophic membrane and the ventricular epithelium. Sometimes they nearly block the lumen and sometimes they distend the ventricular wall. The larger sporonts frequently occupy deep cavities in the epithelium; some of these cavities have been found to extend to the basement membrane, indicating the displacement or destruction of many cells. The shortest life-cycle period determined at 33°C. is eight days. The average number of spores in a cyst is estimated, from partial counts, to be about sixteen hundred.

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